LIFE

Its Nature and Origin

by

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Preface

The mystery of life, so long unsolved, and by many regarded as beyond human understanding, has been slowly revealing itself in the light of scientific investigation. The purpose of this book is to present in coherent order sufficient facts regarding material biological phenomena to show that life is dominated by *catalysis*—the direction of chemical change by surface areas of specific structure and efficiency. Catalysts not only direct the chemical changes essential to life, but some of these catalysts actually are the ultimate living units.

Since matter is the physical basis of life, its successive structural levels are first considered, and then the ways in which atoms and molecules aggregate into masses, and the consequences of the intermixture of substances, even though some are present in mere traces. The nature of living units is next discussed, together with the catalytic mechanism whereby life exists, persists, and proceeds. Immunology, genetics, embryology, diseases and drugs and evolution are then reviewed, and some speculations as to the origin of life are outlined. Since the mental and spiritual aspects of life are quite as real as the material, the last chapter deals with the equally real interrelation between them, even though its basis is unknown.

Each chapter should be considered as part of the whole general picture of life presented, rather than as a separate, isolated entity. Each topic is treated in as simple and elementary a manner as justice to the facts of nature will permit. However, experts in the various fields are aware that no chapter in a book like this can adequately cover their several specialities. It is felt that this outline approach will help the general reader, who is suddenly confronted with so many diverse and puzzling biological problems.

A word about my personal interest in these problems will be found on page 277.

This book represents my reactions to enormous advances in all branches of science for more than half a century. In the attempt to sketch a coherent picture of life processes, I have freely drawn upon the recorded mass of scientific data, with references to origi-

nal publications where feasible. My thanks are due to many publishers, institutions, and individuals for information supplied; acknowledgments for quotations and illustrations are given in appropriate places.

JEROME ALEXANDER

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Introduction

The transformation of non-living matter into the multitudinous forms of living plants and animals, from the visible to the ultra-microscopic, is one of the least comprehensible of all natural phenomena. In this book an attempt is made to explain the basic mechanism of life and its processes in terms of better known and more readily grasped concepts. Life is so usual, so widespread, so persistent, that it peculiarly justifies the motto which hangs on the wall of a large research laboratory:

THIS PROBLEM, WHEN SOLVED, WILL BE SIMPLE

For nature, not limited by our experimental or mental capabilities, solves directly and with ease the most complicated problems submitted to her. Man spends years in endeavoring to discover and comprehend her operative mechanisms, in the hope that he may mold them nearer to the heart's desire.

Though science properly demands that we should accept only what we can unequivocally demonstrate, it is unscientific to condemn a view simply because we cannot check it experimentally with methods at present known. The future has always brought new methods, new light, new insight. Napoleon's clever but sometimes untrue statement—history is a lie, agreed upon—points up the fact that science has sometimes accepted, taught and dignified notions later proven to be erroneous—for example, the indivisibility of the atom, and the belief that all atoms of every element are precisely alike.

The chemist and the physicist, starting with the many kinds of atoms and more recently with the fewer kinds of sub-atomic particles, have been working their way upward into larger aggregation units, the molecules, and into the still larger macro-molecules, chain-molecules, molecular groups, and colloidal particles. On the other hand, biologists in their various fields have been steadily working downward to smaller and still smaller units in the cell and "protoplasm," once naïvely spoken of as "the living jelly." These two groups of investigators have been drilling a tunnel through the mountain of ignorance from opposite directions, and

though the composite terms "biochemistry" and "biophysics" indicate that they have "holed through," it is no simple task to make a perfect joining of the two shafts.

Our endeavor will force us to consider facts in many scientific fields which, for pedagogical convenience, are usually separated into different areas or disciplines, whose frontiers are often jealously guarded or at least respected by teachers. Naturally no attempt is made to give a complete picture of any field or to include every aspect or detail having a bearing on the problem. Since *What is right?* is a more important question than *Who was first?* no attempt will be made to settle questions of priority. Nor is it possible to refer to all pertinent publications; it frequently happens that the same ideas are independently reached by several investigators, and the literature is so vast that reference can be made to only a few publications in the many fields considered.

The last half century has seen such fundamental advances in science that text-books in actual use are often far behind. This institutional time lag requires alertness on the part of teachers if their students are to be kept properly informed. And graduates must remember that no college or university degree stops the progress of science. It is hoped that some of the details included here will prove interesting and helpful to those who, for any reason, have failed to become acquainted with them, or with their interrelations. This interest may stimulate readers to seek out more detailed reviews in the various fields, and thus be led to the multitude of researches reported in scientific journals throughout the world.

Newton, with true humility, said that he was able to see so far because he stood on the shoulders of giants. The real scientific spirit is not exemplified by the few more selfish but less worthy scientists who, basking in the spotlight of ephemeral publicity, forget or ignore what they owe to dead or even to contemporary colleagues. As the great Lord Rutherford so well said in his last address: ". . . it is not in the nature of things for any one man to make a sudden violent discovery; science goes step by step, and every man depends on the work of his predecessors. When you hear of a sudden unexpected discovery—a bolt from the blue, as it were—you can always be sure that it has grown up by the influence of one man on another, and it is this influence which makes the enormous possibility of scientific advance. Scientists are not dependent upon the ideas of a single man, but on the combined

wisdom of thousands of men, all thinking of the same problem, and each doing his little bit to add to the great structure of knowledge which is gradually being erected."[1]

[1] "Background to Modern Science," The Macmillan Co. (N.Y.) and the University Press (Cambridge, Eng.) 1936.

"Books must follow sciences, and not sciences books."—Sir Francis Bacon, "Of Studies".

Contents

Chapter 1

How Did Life Originate?

Regardless of how greatly we may differ as to explanations of its origin, life is an accomplished *fact*. H. E. Richter[1] and Svante Arrhenius,[2] dodged the whole question of the origin of life by suggesting that living spores—"seeds of being," Arrhenius called them—reached the earth from outer space, impelled by "light-pressure" which James Clerk Maxwell had shown to be an important factor affecting the tails of comets. E. Pflüger[3] pointed out the analogies between proteins and cyanogen compounds, and suggested that living matter (protoplasm) arose from cyanogen and other carbon compounds, which formed as the earth cooled. The view is even now commonly expressed that "protoplasm" arose in the "primordial oceanic ooze," when the warm oceans were blanketed by heavy mists and an atmosphere rich in carbon dioxide.

The following definition of protoplasm appeared in "Encyclopedia Britannica:"[4] "A substance, composing wholly or in part all living cells, tissues or organisms of any kind, and hence regarded as the primary living substance, the physical and material basis of life. . . . A living organism of any kind whatsoever may be regarded as composed of (1) protoplasm, (2) substances or structures produced by this protoplasm, either by differentiation of the protoplasm itself, or by the excretory or secretory activity of living substance." The total inadequacy of this definition led me to remark:[5] "Although isolated protoplasm may maintain activity for a short time under suitable conditions, it is incapable of self-reproduction, and should be regarded rather as a highly specific milieu in which the real, living self-reproductive units of cells exist and function. The concept of protoplasm as the ultimate 'living jelly' is a relic of antiquated text-books and should be definitely abandoned. The modern concept of protoplasm embraces the cytoplasm *with* its included nuclear and other particulate units."

"Although the gene is the simplest vital unit definitely known,* it is possible that in some cases the gene itself is an aggregate of simpler units which have been called 'gene elements,' or 'genels.' The hypothetical lower limit of vital units is in the molecular order, for nothing simpler than a molecule, for example an atom, depends for its *increase* in numbers upon forces contained only within the unit itself.

"The question arises: is there in nature any free-living unit of the grade of organization and properties of the gene? It has been suggested that in the bacteriophage and similar ultrafiltrable particles we are dealing with living units (bionts) which either are actually of this degree of simplicity or approach it closely.

"Considering briefly the bearing of these ideas on the origin of life—a very speculative endeavor—it would seem that no biont as complex in its order of aggregation as a cell, or even a bacterium, could have been an initial form of life. The structure of a cell is a box-within-box series of units, of successively simpler orders of aggregation, and it seems reasonable to suppose that the simplest of these units was the first to appear in evolution."[6]

To the primal living unit, the cooling earth was in effect a vast sterile, culture medium, and there existed none of the innumerable and omnipresent mold spores, bacteria, and other contaminants that are now the bane of bacteriologists. On the other hand, while free from competitors and predators, the original biont had at its disposal only such molecules as chance formation gave it.

But matter itself is pregnant with the possibility of life. Once a self-producing unit was formed, its speedy spread was limited only by the then-existing chemical and physical conditions. From such a unit, its later analogs, and their modified forms and ever-varying progeny, there emerged the enormous numbers and varieties of organic molecules now widely available for, and in many cases essential to the life of present-day organisms. Plants and animals surviving under present conditions are adapted to world-wide chemical and physical life with each other, and are in most cases so interdependent that they no longer possess the ability to synthesize all the molecules which they need to live and to propa-

* According to the criteria of "living" given later in this volume, ultrafiltrable viruses and bacteriophages are vital units, and many of them are resolvable in the electron microscope.

gate. In view of changed conditions, if a primal biont were now re-formed, we could hardly expect it to survive.

Considering the origin of life on the basis of mathematical probability alone, the chances that atoms and molecules, in sufficient numbers and variety, would gather together to form the relatively enormous mass and exceedingly complicated structure of an elephant, a man, a flea, or even an ameba, are so remote as to be practically nil. But the *smaller and simpler* the living unit considered, *the greater the probability* that the atomic and molecular units comprising it might have come together by mere chance. The simplest conceivable living unit, or biont, should therefore be considered as the *type* most likely to have begun the wonderful development of plants and animals which teem in sea, air, and earth.[5]

But unrestricted probability calculations cannot be applied to a case like this. We have ample evidence from chemistry and physics that only *some* of the calculable combinations of atoms yield molecules that are both possible and persistent. The work of Svedberg indicates that with larger molecular aggregates, only a few of the many possibilities exist in organisms, the others apparently being impossible or unstable. The physical structure of matter thus weights or "loads" the atomic and molecular "dice" so that only certain combinations actually come into existence. "Out of the inconceivably great number of possibilities in life processes, only relatively few regularly develop, because the results forthcoming are made vastly more probable by factors often going down to the very electronic configurations of atoms themselves."[7]

Such considerations call for a modification of the conclusions which Dr. P. Lecomte du Noüy[8] draws from the probability calculations of Professor Charles-Eugène Guye. Since the original living catalyst was able to initiate its own duplication, there is no need to assume the "compound probability" which plagues du Noüy when he considers the necessity of hundreds of millions of identical molecules.

What is the probability that a well-shuffled deck of cards will be found to have all the hearts, spades, diamonds, and clubs in perfect sequence, and in the suit order named? Vanishingly small, according to mathematical probability calculations. But a like factor of improbability will also apply to *any* particular sequence that actually *does* appear. In the case of our hypothetical primary

living unit, the probability of its appearance is vastly increased by three factors:

(1) the enormous numbers of "card packs" (*i.e.*, mixtures of atoms and molecules) experimented with over the whole earth;

(2) the equally large numbers of "shufflings" of these molecular mixtures over geological epochs, and at high rates of speed;

(3) the inherent structure of material units, which rules out many, if not most, of the theoretically calculable possibilities.

But the *fact* remains that living units *did* arise and undergo evolution in a most marvelous manner, even though scientists, gradually emerging from ignorance, are unable in their few human generations to understand all the biological details of the past, much less to duplicate them experimentally. We have not yet discovered a synthetic medium in which to grow viruses or even the leprosy bacillus, although the latter is closely related to the tubercle bacillus which grows well in Long's synthetic medium.

The probability that any living unit will originate spontaneously increases enormously with its simplicity. Since we know neither the nature of the hypothetical first living unit, the nature of the then-existing milieu, nor the value of the three factors mentioned above, we have slight basis for calculating the probability for the chance formation of such a unit. We cannot, even in imagination, construct a complete picture of terrestrial conditions when life first appeared, say a billion or more years ago. The composition of the earth's atmosphere; solar radiation and its ability to reach the earth's surface; the alpha, beta, and gamma radiations from radioactive atoms—all must have been quite different from what they are at present. Other factors may be unknown or unweighted; for instance, fourteen hundred million years ago the amount of uranium isotope 235 was about four times what it is now.

When once formed, a "living molecule" could serve as a mold or template for the production, not only of incalculable numbers of like molecules but also of new types arising from modifications of the older ones. The initial absence of predators must have been a most favorable factor; for though the spontaneous generation of living units may even now be continually occurring at the ultramicroscopic level, existing and well-established forms of life make their chance of persistence very slight, either by devour-

ing them or by depriving them of nourishment. Furthermore, the chance of locating such a new living unit in this low size bracket, of perhaps transient existence, and at unknown place of origin, would be very slight.

If a new or a modified self-duplicating agency arises in a cell, it may persist there, and its presence may lead to chemical consequences which are beneficial to the cell or to the organism of which the cell is part. If it is carried on to succeeding generations, it may result in evolutionary advance or retrogression (see Chapter 11). Where the chemical consequences of the new agency are harmful to the cell or its organism, they may cause disease or death (see Chapter 10). The cell or its organism may respond by forming a specific antibody which will destroy or inhibit the new agent or its harmful products, just as may happen to any antigen (see Chapters 7 and 8).

The formation and establishment of a new or a modified self-duplicating mechanism in any cell or organism is, in effect, the creation of a new living unit at or near the molecular level. Such a process may well be of common occurrence, even though the new units thus formed may not be able to survive if removed from the special milieu in which they appear. Units like mitochondria and viruses may thus be formed *de novo*, even though they may also be formed by the degeneration of parasitic or symbionic invaders, along the lines suggested by Prof. Robert H. Green. But whatever was the structure of the original living unit which initiated life on this earth, it must have been able to start life and to persist on the basis of what inanimate nature could then supply. The origin of new units, or the modification of some of the existing units, would greatly add to the evolutionary possibilities.

Professor Michael I. Pupin of Columbia University thus concluded an address at a dinner in honor of Sir James Hopwood Jeans, Professor of Astronomy at The Royal Institution:[9]

"Science cannot penetrate the mysterious veil which covers the face of the space-time entity, separating the world of ultimate reality from the world presented to our senses and interpreted by mere pictures of mathematical symbols. Faith alone penetrates it and finds behind it the throne of the divinity which created that space-time entity and filled it with electrons and protons, and with their offspring, the omnipresent photons, the tiniest and liveliest energy granules in the ever-expanding interstellar space.

One cannot resist the hope that some day, perhaps, we may discover that these ultra-refined energy granules are responsible for the first beginning of life and for its never-ending evolution."

But Professor Pupin offered no suggestion as to the kind of mechanism whereby photons could initiate life. The most reasonable view, in the light of present knowledge, is that life began with the chance formation of a self-reproducing unit of molecular or near-molecular complexity.

Faith

What most persons consider the greatest of all human problems, survival after death, is one to which we have no direct experimental approach. We treasure memories, mementoes and pictures of those we have loved and lost; we dream of them and yearn for them, recalling the poignant line of Virgil:

*Tendebant manus, ripae ulterioris amore.**

But we face the tragic and inexorable fact so concisely stated by Omar:

Strange, is it not, that of the myriads who
Before us pass'd the door of Darkness through,
Not one returns to tell us of the Road,
Which to discover we must travel too?

It is interesting to note that Edgar Allan Poe's "The Raven," which depicts the torturing doubts of a bereaved husband, stimulated Dante Gabriel Rossetti to write "The Blessed Damozel," which stresses faith in reunion.

Faith involves no compelling necessity for believing that divine interposition guides every detail of mental and material activity. Whatever occurs in nature was already inherently possible when matter came into being. Tyndall said: "I see in matter the potentiality and possibility of all life." This view simply pushes back the mysteries of life to the creation of matter, a still more remote mystery not open to experimental attack. But no one doubts the existence of matter, the forces affecting it, and the fact that living, thinking beings are continually emerging from it. Yet it requires even more faith to believe in the reality of matter, which we know of only indirectly through our senses, than it does to believe in

* "They stretched their hands, yearning for the farther shore." (*Aeneid*, Book VI).

the reality of our mind, with which we are in much more direct contact.

Just as physicists have been forced to have faith in the existence of unobservables, as being in the nature of things, so we are forced to have faith in the reality of "unobservable"* mental and spiritual phenomena. This philosophical point of view has, of course, nothing in common with numerous mercenary frauds, or with delusions based on wishful thinking, or with "mediums," "psychics," "spiritualists," and even some "religions."

Applying Herbert Spencer's criterion of truth—the inconceivability of the opposite—we are led by faith to believe that life has some future significance and meaning, even though we are and must be ignorant of just what it is. Nothing is more real to us than our own personality, even though we cannot understand its basis. Most religions and creeds have been formed about this basic kernel of faith, which, despite uncertainties and fears, remains mankind's greatest comfort; for it involves the hope and expectation that we shall, in some way, admittedly unknown, be reunited with those we love. Some accept faith in the persistence of mind or soul from formal creeds; others are led to this view by philosophical consideration of the material and spiritual facts of life. Nevertheless, despite differences of opinion as to matters within our control, and as to those at present beyond it, we carry on to serve our living fellows. The following lines expressing this view have been called "A Scientist's Psalm":

Almighty Power! Too vast to be
Compassed by human mind or hand,
With loving awe we reverence Thee,
Striving to see and understand.

Within the atom's ordered maze,
Earth's lumined book, writ to be read,
Beyond the star-dust's far flung haze,
We seek Thy works with joy, not dread.

Our souls, which by Thy richest grace,
Have waked to justice, mercy, love,
Find in humanity Thy face,
And serving men, serve Thee above.

* Perhaps they should be termed "directly observable."

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[1] "Zur Darwin'schen Lehre," in Schmidt's *Jahrb. d. ges. Med.* (1865) **126,** 243; "Bericht über medicinische Meteorologie und Klimatologie," *ibid.* (1870) **147,** 57; "Die neueren Kenntnisse von den krankmachenden Schmarotzerpilzen," *ibid* (1871) **151,** 313.

[2] "Worlds in the Making," 1908, pp. 212-231.

[3] "Ueber die physiologische Verbrennung in den lebendigen Organismen," *Arch. ges. Physiol.* (1875) **10,** 251.

[4] 11th ed. (1916) **21,** 477.

[5] *Scientia,* Oct., 1933, p. 253.

[6] Paper presented before the American Association for the Advancement of Science Dec. 28, 1928 [*Science* (1929) **70,** 508-10] by J. Alexander and C. B. Bridges.

[7] "Colloid Chemistry," Vol. II, p. 1, New York, Reinhold Publishing Corp., 1928.

[8] "Human Destiny," p. 34, Longmans, Green & Co., 1947.

[9] *Scientific Monthly,* July, 1931, p. 11.

"A little philosophy inclineth man's mind to atheism, but depth in philosophy bringeth men's minds about to religion."—Sir Francis Bacon, "Of Atheism".

Chapter 2

The Smallest Particles of Matter

Until comparatively recently, atoms were thought to be the smallest units of matter. In fact, the word *atom*, derived from the Greek, literally means "that which cannot be cut." About 380 B.C. Greek philosopher Democritus, allowing his imagination to outrun his senses and all means of observation then known, maintained that infinite space is populated by an infinite number of atoms which he conceived to be eternal, homogeneous, invisible, and indivisible. The smooth, round atoms of water were supposed to roll readily past each other, whereas the jagged atoms of iron hooked tightly together. Democritus regarded the soul as consisting of round, smooth and exceptionally mobile atoms which in the head, control reason; in the heart, anger; in the liver, desire. Life, he thought, requires the inhalation of fresh atoms to replace those lost by exhalation; so that when respiration ceases, death results, and the soul perishes with the body.

To explain nature, Plato and Aristotle invoked human reason and reactions, rather than matter and experiment. Aristotle considered that four primary qualities—hotness and coldness, wetness and dryness—combined in pairs to form the four "elements," earth, air, fire and water. These "elements," described essentially by human reactions, united in various proportions to make up various kinds of matter.

The atomic views of Democritus were temporarily revived by Epicurus (341-270 B.C.) and Lucretius (95-51 ? B.C.); but during the Middle Ages the ideas of Aristotle prevailed, even though his four elements competed with a subsequent alchemistic notion that salt, sulfur and mercury are the basic "principles" or "essences." Thus Chaucer (1340-1400) wrote of the Clerk of Oxenford:

For hym was levere have at his beddes heed
Twenty bookes clad in blak or reed
Of Aristotle and his philosophy,
Than robes riche or fithele or gay sautrie.

Faith in experiment developed, however, and Leonardo da Vinci, Copernicus and Galileo are among the predecessors of Robert Boyle (1627-1691) who, in his book "The Sceptical Chymist" (1661), espoused the view that unalterable atoms exist, which survive in various chemical combinations.[1]

The Electron

Sir William Crookes' electric experiments with vacuum tubes, together with the work of Hittorf, Goldstein, Varley, and others, helped develop the concept of radiant energy as a "fourth state of matter." This resulted in the modern concept of the electron, a term suggested in 1891 by Dr. G. Johnstone Stoney for the natural unit of electricity to which he had called attention in 1874.[2]

The name *electron* was later applied to subatomic particles having negative electric charges. Sir J. J. Thomson (Nobel prize, 1891) first called them "corpuscles," when in 1897 he proved them to be constituents of cathode rays, so named by Goldstein in 1876. This led to the conception of an electric current as a stream of electrons.

Professor Robert A. Millikan (Nobel prize, 1917) actually weighed single electrons and showed that they are all of identical mass—that is that they all contain the same quantity of matter. In principle, his method is quite simple. He caught electrons on tiny ultramicroscopic oil droplets, and observed how much faster a droplet would fall in still air when one or more electrons were added to the drop. On some droplets he had as many as 150 electrons, stuck like currents on a bun, and each of them added the same increment of fall. Millikan's "electrical balance" could weigh accurately and easily to one ten-billionth of a milligram, whereas the quartz-fiber balance of Ramsay and Spencer, in a vacuum, was 10,000 times less sensitive. The most delicate chemical balance of fifty years ago could weigh only about 1/100 milligram—for example, your pencilled signature on a card. According to Millikan's estimate, the number of electrons contained in the 1/100,000 of a cent's worth of electricity passing in one second through a 16-candlepower carbon-filament incandescent lamp is so enormous that if the (then) 2½ million inhabitants of Chicago were to count them at the rate of two per second, and were to keep counting steadily day and night, they would take 20,000 years to finish the count.

Roentgen and X-rays

In 1895 Professor Wilhelm Konrad Roentgen (Nobel prize, 1901) of Würtzberg found that when the cathode rays in a Crookes

tube strike a metal target within the tube, an intensely penetrating form of radiant energy emerges. This is known as x-rays or Roentgen rays; they cause fluorescence in many substances, and affect photographic plates. I well remember the thrill we students got when, soon after Roentgen's discovery, Dr. L. H. Friedburg showed us a photograph of the bones of his hand which he had taken by means of x-rays emitted by one of the Crookes tubes in the laboratory of Professor R. Ogden Doremus at the College of the City of New York.

Becquerel and Radioactivity

Important consequences followed quickly upon Roentgen's discovery. Professor Henri Antoine Becquerel of Paris (Nobel prize, 1903) had developed an interest in fluorescence and phosphorescence from his father, also a professor at Paris. In 1880, he had prepared crystals of the double sulfate of potassium and uranium, which glow beautfully when exposed to light. To see what connection there might be between phosphorescence and x-rays, Becquerel wrapped a number of phosphorescent substances in ample folds of black paper and placed them on photographic plate holders. No effect was registered. Recalling his uranium salt, he tried some that had been illuminated, and obtained a noticeable photographic effect, which also appeared when he interposed a thin glass plate to stop possible vapors. At first Becquerel thought that this was due to phosphorescence; but later he found that the photographic image was just as marked when the uranium salt had been kept for weeks in a dark drawer. He also noted that, if uranium salts are placed near a charged gold-leaf electroscope, the uranium rays ionize the air and increase its electrical conductivity. As a result, the charge leaks away more rapidly and the strips of gold leaf quickly fall together. Becquerel thus discovered the phenomenon of *radioactivity*, and this had prompt and extremely important repercussions in many fields of science.

The Curies and Radium

Marie Skladovska Curie (Nobel prize, 1903 and 1911), while working for her Doctor's degree in Paris, began measuring with a gold-leaf electroscope the relative radioactivity of various uranium salts. She quickly found that the radioactivity is proportional to the amount of uranium present, irrespective of its mode of chemical combination. This indicated that radioactivity is property of *atoms*. Thorium, too, was later found to be radioactive. For-

tunately, Mme. Curie *also tested the original ores* (pitchblende and carnotite) from which uranium is extracted. To her astonishment, they showed much *greater* radioactivity than pure uranium. The conclusion was obvious: the ores must contain some substance or substances more strongly radioactive than uranium.

With her husband, Professor Pierre Curie (Nobel prize, 1903) she began the exhausting task of trying to isolate this important "impurity." Their first paper (1898)[3] stated: "We believe the substance we have extracted from pitchblende contains a metal not yet observed related to bismuth in its analytical properties. If the existence of this new metal is confirmed we propose to call it *polonium* from the name of the original country of one of us."

A few months later came the announcement of *radium*, the radioactivity of which is actually about 2,000,000 times that of uranium. The Curies wrote: "The new radioactive substance contains a very strong proportion of barium; in spite of this its radioactivity is considerable. The radioactivity of radium therefore must be enormous."

To show the difficulties overcome by these great French scientists in making their epoch-making discoveries, the following is quoted from "Madame Curie—A Biography," by Eve Curie, her daughter:[4]

"It was necessary, of course, to buy this crude material and to pay for its transportation to Paris. Pierre and Marie appropriated the required sum from their very slight savings. They were not foolish enough to ask for official credits. . . . If two physicists on the scent of an immense discovery had asked the University of Paris or the French Government for a grant to buy pitchblende residues they would have been laughed at. In any case their letter would have been lost in the files of some office, and they would have had to wait for months for a reply, probably unfavorable in the end. Out of the traditions and principles of the French Revolution, which created the metric system, founded the Normal School, and encouraged science in many circumstances, the State seemed to have retained, after more than a century, only the deplorable words pronounced by Fouquier-Tinville at the trial in which Lavoisier was condemned to the guillotine: 'The Republic has no need for scientists.' "

Rutherford and Radiations

In 1895 Ernest Rutherford began work in the Cavendish Laboratory at Cambridge (England) on the ionization of gases by x-rays. Reading of Becquerel's work, he made a systematic exami-

nation of uranium radiations, and found that there are two types: (1) alpha rays, now known to be helium nuclei, which produce intense ionization but are absorbed in a few centimeters of air; (2) beta rays, now known to be high-speed electrons, which produce less ionization, but are more penetrating. The still more penetrating gamma rays, now known to be "hard" or short-wave length x-rays, were discovered by Villard in 1898. Rutherford, then at McGill University in Montreal, found that thorium gives off a material "emanation," which passes through paper but is held back by a thin sheet of mica. When in contact with substances, this "emanation" made them radioactive. Radon, the gaseous emanation from radium, is nowadays collected in tiny tubes which may be inserted into cancerous growths; it loses half of its radioactivity in 3.823 days and emits alpha particles at about 5½ million electron-volts.

Ramsay and Helium

A few years later (1903) Sir William Ramsay (Nobel prize, 1904), who had discovered the inert rare gases of the atmosphere (argon, krypton, xenon, neon, now used in red electric lamps, and helium, identified in the sun by Sir Joseph N. Lockyer in 1868), showed that helium is being continuously formed in radioactive minerals in amounts that can be recognized in a spectrograph, and that it is another product of radioactive transformations. Alpha particles are helium nuclei carrying two positive charges.

The rare gases of the atmosphere had actually been isolated by the distinguished chemist and physicist Henry Cavendish (1731-1810), who demonstrated the composition of air in 1793 and of water a year later. But he failed to discover why a tiny residue of "nitrogen" could not be oxidized: it consisted of the rare atmospheric gases. Much later the distinguished American chemist, W. F. Hillebrand,[5] in the course of his analysis of the mineral Cleveite, a variety of uraninite rich in uranium oxide, isolated a small quantity of a gas which, from its inertness, he assumed to be nitrogen. Spectrographic examination would have proved it to be helium, as Ramsay later found. Actually, Dr. A. P. Hallock, later Professor of Physics at Columbia University, did make a spectrographic examination of Hillebrand's "nitrogen" and observed in it the bright yellow spectral lines of helium. But he, too, was outfaced from his prize by the skepticism then prevailing against "new elements." Dean George B. Pegram, Professor of Physics at Columbia University, informs me that Professor Hallock often spoke

with philosophic chagrin of his failure to insist on the significance of his finding.

All the evidence indicated that radioactive atoms are continuously and spontaneously decomposing—giving off material particles and powerful radiations; but the precise origin of the matter and the energy was still unknown.

Soddy and the Periodic Table of the Elements

Frederick Soddy (Nobel prize, 1921) investigated the chemical properties of the substances released by successive radioactive transformations, and observed a simple relation, in the Periodic Table of the elements constructed by Mendeléef and Newlands, between the position of the original and the final elements involved in a radioactive disintegration series. Almost simultaneously Dr. A. S. Russell, Professor K. Fajans, and Dr. Soddy announced the "displacement law": when a substance emits an alpha particle (helium nucleus) *it moves two places down* in the atomic table; but it *moves one place up* when it emits a beta particle (electron). Later on, we shall see the importance of this in the transformation of uranium 238 into neptunium and plutonium.

In 1906 Boltwood observed that his newly discovered radioactive element ionium (atomic weight 230) was chemically so similar to radium (atomic weight 226), that he was unable to separate their salts, once they were mixed. On the basis of this and similar facts, Soddy stated (1910): "These regularities may prove to be the beginning of some embracing generalization, which will throw light not only on radioactive processes, but on elements in general and the Periodic Law . . . Chemical homogeneity is no longer a guarantee that any supposed element is not a mixture of several different atomic weights, or that any atomic weight is not merely a mean number." Professor Theodore W. Richards of Harvard University (Nobel prize, 1914), in making extremely accurate determinations of atomic weights, had found marked differences in the atomic weights of lead from different sources. Soddy further wrote: "The same algebraic sum of the positive and negative charges in the nucleus when the arithmetical sum is different, gives what I call 'isotopes' or 'isotopic elements,' because they occupy the same place in the Periodic Table. They are chemically identical, and save only as regards the relatively few physical properties which depend upon atomic mass directly, physically identical also."[5a]

Emptiness in the Atom

In connection with cathode-ray experiments conducted with Heinrich Hertz of radio-wave fame, Phillip Lenard (Nobel prize, 1905) observed that cathode rays could pass through a "window" of thin aluminum foil and still retain sufficient energy to cause fluorescence and phosphorescence. Lenard concluded that the atoms in the foil must have a very open structure, and also that they might have localized positive and negative electric charges. Sir J. J. Thomson, in developing this idea, calculated how negative electrons would be distributed in a sphere of positive charge, a matter of importance in understanding the Periodic Table. Professors William Draper Harkins (Chicago), Gilbert N. Lewis (California), and Dr. Irving Langmuir (Nobel prize, 1928), also contributed much to establishing how electrons are distributed in atoms.

Meanwhile, Rutherford had been investigating scattering of alpha particles by atoms; but his assistant, Dr. E. Geiger, had observed only small scattering (about one degree) in thin pieces of heavy metal. However, Rutherford set Geiger and a young student, E. Marston, the task of finding out whether any alpha particles could be scattered through a large angle by impact upon an atom. Within a few days, to their great astonishment, they observed some alpha particles *coming backward.* In a lecture given shortly before his death, Lord Rutherford said:

"It was quite the most incredible event that has ever happened to me in my life. It was almost as incredible as if you fired a 15-inch shell at a piece of tissue paper and it came back and hit you. On consideration I realized that this scattering backwards must be the result of a single collision; and when I made calculations I saw that it was impossible to get anything of that order of magnitude unless you took a system in which the greater part of the mass of the atom was concentrated in a minute nucleus. It was then that I had the idea of an atom with a minute massive centre carrying a charge. I worked out mathematically what laws the scattering should obey, and I found that the number of particles scattered through a given angle should be proportional to the thickness of the scattering foil, the square of the nuclear charge, and inversely proportional to the fourth power of the velocity. These deductions were later verified by Geiger and Marsden in a series of beautiful experiments."

The extent of the "wide open spaces" in atoms is shown in the following comparison: If the tiny proton nucleus of a hydrogen

atom be represented by a period on this printed page, its satellite electron would be represented by a small pea about 50 miles away. Some notion as to the immensity of the numbers of atoms and molecules is given by this estimate of Professor Herbert Freundlich: Imagine each water molecule in a liter of water marked for identification, and the whole lot uniformly mixed throughout all the waters of all the seas and oceans, down to the lowest depths. Suppose, thereafter, that a liter of water is taken at random, anywhere in the oceans of the world; it would contain approximately 25,000 of the original marked molecules.

Isotopes—a New Concept of the Elements

Shortly before World War I, Sir J. J. Thomson developed the mass spectrograph, an instrument in which positively charged ions of elements (or of compounds) in motion are subjected to the pull of magnetic or electric fields and are diverted from their course in proportion to their mass. On examining (1912) the rare gas neon, he found that it gave two parabolas, one corresponding to particles with a mass of 20, the other to particles with a mass of 22; that is, neon has two kinds of atoms. In 1919 Francis William Aston of Cambridge University (Nobel prize, 1922) using improved apparatus, confirmed Thomson's work and found that most elements have atoms of different atomic weights, though all atoms of the same element have the same chemical properties.

Thus fell another dogma of chemistry—the notion that all atoms of each element are precisely alike. Even in the cases of the relatively few natural elements where this is so (*e.g.*, beryllium, fluorine, sodium, argon, phosphorus, manganese, cobalt, arsenic, iodine, molybdenum, and gold), new man-made radioactive isotopes, usually of transient life, have been created.

Some of our natural elements have quite a number of isotopes, all chemically the same because they have the same net positive nuclear charge and therefore the same number of planetary electrons; but the masses of the isotopes may differ considerably. It is the exterior electron rings which determine the chemical properties of an atom, recalling the saying that beauty is only skin deep. In the case of the metal *tin*, nine radioactive isotopes have been made synthetically, with half-lives running from 9 minutes to 400 days; but the *natural* metal consists of ten isotopes whose atomic weights range from 112 to 124.[6] Chemists use the average, 118.70 as the atomic weight of tin; for though the mass of the individual atoms varies, the natural average is practically constant, and all the atoms act chemically like tin.

The question of isotopes is no longer academic. In order to make the first of the "atomic bombs" tested at Alamogordo, New Mexico, and later used at Hiroshima, the fissionable uranium isotope 235 had to be separated from the non-fissionable isotope 238 by an ingenious application of the process of differential diffusion of uranium hexafluoride. This work was based on the pioneer diffusion experiments of Thomas Graham, in the course of which he coined the word "colloid" and established much of the nomenclature of colloid chemistry. The bomb dropped on Nagasaki was of the new fissionable material plutonium, synthesized (indirectly) by bombarding the isotope U^{238} with neutrons.

Another outstanding case of the importance of isotopes is found in our lightest element, hydrogen. The discovery by Professor Harold C. Urey (Nobel prize, 1934) that common water contains a "heavy" fraction, was quickly followed by the isolation of deuterium, whose atomic weight is double that of hydrogen. Natural hydrogen contains 0.02 per cent of deuterium, whose nuclei contain one proton and one neutron, and have a net nuclear charge of one. Triple hydrogen, called tritium, with a radioactive half-life of about 30 years, has been artificially produced; it may be readily stored by combining it with lithium as lithium tritide.

Moseley and X-ray Spectra

Henry G. Moseley was killed at Gallipoli in 1915 at the age of 28 as a result of the unwise drafting of highly trained scientists; his death was both a calamity and a disgrace. He had already brilliantly begun investigating the relation between nuclear charge and atomic number—the property which fixes the position of an element in the Periodic Table. Moseley used the crystal spectrometer developed by Sir William H. Bragg and his son, Sir Lawrence Bragg, who jointly received the Nobel prize in 1915. A direct correlation was discovered between the wave lengths of x-rays emitted where different metals were used as targets in the x-ray tubes, and the atomic number of the metal. The x-ray spectra varied uniformly and regularly, all the lines being similar; but they shifted in frequency in passing from one element to the next, because they all depend upon the number and arrangement of the planetary electrons circulating about the nucleus, whose net positive charge Moseley supposed to be identical with the atomic number. This was later proved by Sir James Chadwick (Nobel prize, 1935).

Then Dr. Niels Bohr (Nobel prize, 1922) developed his quantum theory of spectra, referred to by Rutherford as one of the greatest triumphs of the human mind. Within ten years all the main features of optical spectra were understood, largely helped by the application of wave mechanics by Werner Heisenberg (Nobel prize, 1932), Erwin Schrödinger (Nobel prize, 1933), and P. A. M. Dirac (Nobel prize, 1933).

Rutherford and Atomic Transmutation

In 1919 Rutherford made the astounding discovery that when light atoms are bombarded by alpha particles shot out at very high speeds from radioactive substances, scintillations could be detected in a device called a "cloud chamber." When nitrogen was the gas bombarded, these scintillations struck out 40 centimeters or more. By subjecting these new "rays" to the action of a magnetic field and observing their deflection, Rutherford found that they had the mass and charge of protons—that is, they were hydrogen nuclei. His explanation was that when the helium nucleus (alpha particle) enters the nucleus of a nitrogen atom, the nitrogen nucleus is forced to eject a proton. The final result is a nucleus with a positive charge of 8 and a mass of 17—one of the isotopes of oxygen.

Professor Harvey E. White writes:[7] "The discovery of the disintegration and transmutation of stable elements by controlled experiments is attributed to the great experimental genius, Sir Ernest Rutherford. Outsiders might say the discovery was an accident, but to those who knew him well it was the result of a long series of well-planned experiments. True, he did not predict the phenomenon and then discover it, but his long experience with radioactivity and his keen insight enabled him to recognize the meaning and importance of the phenomenon when it was first observed. Due credit must also be given to the admirable work of his collaborators and to experimenters in other laboratories who have since carried the work much further."

And I cannot refrain from repeating here what some scientific wag wrote of Rutherford:

He made plain the invisible;
He broke up the indivisible;
He changed the immutable;
And he unscrewed the inscrutable.

Professor William D. Harkins and his collaborators, who were for fifteen years (1913-1928) the only scientists in America engaged in work on the structure of the atomic nucleus, advanced the idea that an intermediate compound nucleus of short life was formed by the impact of the alpha particle.[8] He came to this conclusion soon after Rutherford's 1919 experiment, and represents the transmutation of N^{14} into O^{17} by the following diagram:

The almost incredible density of atomic nuclei appears from the following quotation from Harkins' paper: "The electron, or elec-

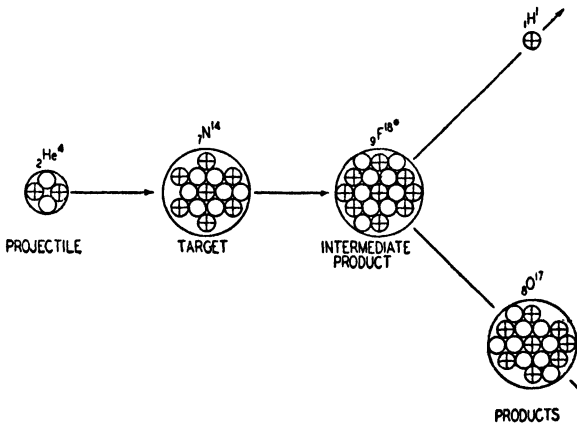

FIGURE 1. Formation of oxygen 17 and hydrogen 1 by the disintegration of fluorine 18, the intermediate nucleus formed by the impact of helium 4 with nitrogen 14. Open circles=neutrons. Circles with plus signs=protons. At higher energies the excited fluorine nucleus disintegrates in two ways: (1) that given in the diagram, and (2) to give a neutron and positive electron in place of a proton (=hydrogen 1). [Courtesy *Science*, Vol. 103, No. 2671 (1946)]

trons, may be considered to move around the nucleus, where they form a cloud which is exceedingly tenuous, when considered in comparison with the high density of the nucleus. The mass of the proton is 1.672×10^{-24} gram. If this is contained in the 1.4×10^{-38} cc. considered as its volume, the density is 1.2×10^{14} grams, or 130 million tons per cc. Thus, the whole earth would have a diameter of only 460 meters, or less than a third of a mile (0.286 mile) if its *whole mass* were present as protons and neutrons packed as they are in the nucleus of an atom. The presence of electrons, however, reduces the density from 1.2×10^{14} g. per cc. to that of the earth (5.522 g. per cc.) which is 20 million million times smaller."

Neutrons and Positrons

Though both Rutherford and Harkins independently assumed as early as 1920 that the neutron exists, it was not discovered until

1932 by Sir James Chadwick (Nobel prize, 1935). This opened the way for understanding how the heavy proton and the agile electron can be associated with still other particles in atomic nuclei. In 1932 Carl David Anderson (Nobel prize, 1936) discovered the positron, or "positive electron." We have as yet no complete explanation of how, under sufficient impact or excitation, electrons (beta rays) and positrons may emerge from atomic nuclei. Rutherford suggested that within the confines of the nucleus where particles are at close grips and subject to enormous forces, protons may change to neutrons, and *vice versa.* P. A. M. Dirac (Nobel prize, 1933), predicted that if a high frequency gamma ray (a high-energy photon) were to pass near enough to an atomic nucleus, the powerful nuclear field would "annihilate" the gamma ray, and "create" a pair of particles, an electron and a positron. This phenomenon, known to physicists as *pair production,* was soon observed by P. M. S. Blackett and others.[9]

Joliot and Induced Radioactivity

In 1934, Jean Frederick Joliot and his wife Irene Curie-Joliot (Nobel prize, 1945) while bombarding aluminum with alpha particles from polonium, found that even after the polonium emitter had been removed, the aluminum still continued to emit radiation—that is, it had become radioactive. What happened may be understood from the following:

$$\begin{array}{l} {}_2He^4 + {}_{13}Al^{27} \longrightarrow {}_{15}P^{30} + {}_0n^1 \\ \qquad\qquad\qquad\qquad\quad \searrow \\ \qquad\qquad\qquad\qquad\qquad {}_{14}Si^{30} + {}_{-1}e^0 \end{array}$$

The entering alpha particle had changed the aluminum into an unstable isotope of phosphorus with the ejection of a high-speed neutron; and the phosphorus nucleus (half-life 2.55 min.) gradually changed into a stable isotope of silicon. On dissolving the radiated aluminum in HCl, adding some ordinary inactive phosphorus-containing salt, and then carrying out an ordinary analytical group separation, the radioactivity followed the phosphorus.

Ernest O. Lawrence and the Cyclotron

In 1932, Professor Ernest O. Lawrence of Univ. of California (Nobel prize, 1940) announced the cyclotron. Hundreds of synthetic isotopes have been recognized, and these have been mainly produced by use of the cyclotron. Following the announcement

of the Curie-Joliot discovery, Lawrence bombarded sodium with 2 mev (million electron-volt) deuterons, from his cyclotron, and found that it became radioactive. The radio-sodium formed has a half-life of only 15 hours; it changes into a stable isotope of magnesium, giving off an electron and a gamma ray.

When a high-speed deuteron from the cyclotron crashes into a beryllium nucleus, an unstable, radioactive isotope of boron is formed, which becomes a stable isotope of boron by ejecting a neutron of 21 mev energy. Unstable nuclei are also capable of undergoing *multiple* and *branch disintegrations.* For example, when boron is bombarded by protons it forms an unstable carbon nucleus, ${}_6C^{12}$, which breaks down in steps into three helium nuclei; and on bombarding lithium with deuterons, the unstable ${}_3Li^6$ formed may break down either into ${}_3Li^7 + {}_1H^1$, or into ${}_2He^4 + {}_2He^4$.

Professor Glenn T. Seaborg (California) has described[10] the transuranium elements thus far produced, as well as the astonishing ultramicrochemical methods used in following the work, including the use of quartz fiber microbalances with a sensitivity of 0.02 μg. The new elements are neptunium (Np^{239}, half-life 2.3 days), plutonium (Pu^{239}, half-life 24,000 years), americum (Am^{95}, half-life 500 years) and curium Cm^{96}, half-life of isotope 240, one month; of isotope 242, five months). Both neptunium and plutonium have other isotopes than the main ones above indicated. On Aug. 18, 1942 the first pure chemical compound of plutonium was produced, and Seaborg states: "This memorable day will go down in scientific history to mark the first sight of a synthetic element and the first isolation of a weighable amount of an artificially produced isotope of any element."

Enrico Fermi and Nuclear Fission

Shortly after Chadwick had discovered the neutron, Professor Enrico Fermi (Nobel prize, 1938) made a number of new radioactive isotopes by exposing various elements to this uncharged missile. Usually the nucleus would capture the neutron, and often the atom would return to a stable state by emitting a beta ray, thereby yielding an element having an atomic number one unit higher in the Periodic Table than the parent atom. Fermi wanted to see what would happen in the case of uranium, the last element in the atomic table with an atomic number of 92. After prolonged exposure to neutron bombardment, the activity showed the existence of particles with *four* half-lives, and indications of others; but natural uranium has only *three* isotopes. This led to the notion that one of the supernumerary activities might be due

to a newly formed transuranic element 93, which ought to appear in the Periodic Table in the same column with manganese. So the irradiated uranium was dissolved with a manganese salt, and the manganese was precipitated as MnO_2. This precipitate carried with it part of the 13-minute and 90-minute activity, and many scientists took up the quest for transuranic elements.

In Germany, Otto Hahn (Nobel prize, 1946) and F. Strassman made careful chemical separations of the dissolved uranium, and were astonished to find radioactive atoms belonging to a number of different elements, mostly those about the center of the Periodic Table. These results were passed to Professor Niels Bohr, then at Princeton University, reaching him at a meeting of the American Philosophical Society; and many nuclear physicists who attended the meeting confirmed the results.[11] Professor Henry D. Smyth states:[12] "Just before Bohr left Denmark, two of his colleagues, O. R. Frisch and Liese Meitner (both refugees from Germany) had told him of their guess that the absorption of a neutron by a uranium nucleus sometimes caused that nucleus to split with the release of enormous quantities of energy, a process that soon became known as 'nuclear fission.' " Before considering further details about the "atomic bomb," let us see whence comes the huge amounts of energy released by nuclear fission.

Albert Einstein and the Relation Between Mass and Energy

About the end of the last century, Hendrik Antoon Lorentz (Nobel prize, 1902) in developing an electron theory of matter, assumed that the mass of an electron increases with increase in its velocity;[12a] but if the velocity could be equal to the speed of light (186,000 miles a second) the mass would be infinite. Therefore, no material body, as we know matter, can travel with the speed of light.[13] Measurements on electrons emitted at high speeds from radioactive elements indicate that those with velocities of 1 per cent of the velocity of light (*i.e.*, 1,860 miles per second) show no appreciable increase in mass; at 50 per cent of the velocity of light the mass increases 15 per cent, while at 99 per cent of the velocity of light the electron showed more than seven times its mass when at rest.

Albert Einstein (Nobel prize, 1921) derived from his restricted theory of relativity an equation similar to that of Lorentz. Einstein also derived what is known as the *mass-energy equation*, $E=mc^2$, where E is the energy equivalent of mass, c the velocity

of light, and m the mass. This means that if one pound of matter *could be entirely converted into energy*, it would yield $1 \times (186{,}000 \times 5{,}280)^2$ foot-pounds of energy, or nearly $11\frac{1}{4}$ *billion* kilowatt hours of energy, an amount nearly equal to the total monthly output of the electric power industry in the United States as of 1939. In the fission of the U^{235} nucleus it is estimated that only one-tenth of one per cent of the total mass-energy is released, *i.e.*, $11\frac{1}{4}$ *million* kilowatt hours per pound.

Experimental Proof of the Equivalence of Mass and Energy

Proof that Einstein's equation satisfactorily expresses the relation between mass and energy is concisely stated by Professor H. D. Smyth[12] about as follows: Gradual improvement in high-voltage technique made it possible to substitute artificially produced high-speed ions of hydrogen or helium for natural alpha particles. J. D. Cockcroft and E. T. S. Walton in Rutherford's laboratory were the first to succeed in producing nuclear changes by such methods. In 1932 they bombarded a target of lithium with protons of 700 kilovolts energy and found that alpha particles were ejected from the target as a result of the bombardment. The nuclear reaction which occurred can be written symbolically as

$$_3Li^7 + {}_1H^1 \rightarrow {}_2He^4 + {}_2He^4$$

where the subscript represents the positive charge on the nucleus (*atomic number*, generally represented by Z, the number of nuclear protons), and the superscript is the number of *massive* particles in the nucleus (*mass number*, which is the sum of the number of protons *and* neutrons, but which omits the tiny masses of any electrons, positrons, or other particles which may be present).

The above equation, like most chemical equations, does not include anything relative to mass or energy, but merely indicates a redistribution, in nuclei, of protons and neutrons. Since both mass and energy are indestructible, their sums should be the same before *and* after the reaction. The masses of Li^7 and H^1, as determined from mass spectra, total 8.0241, while the mass of $_2He^4$ is 8.0056. Therefore 0.0185 unit of mass "disappeared" in the reaction.

The experimentally determined energies of the alpha particles ($_2He$) was approximately 8.5 million electron-volts *each*—a figure so great that the kinetic energy of the incident proton which caused the change may be neglected. That is, 0.0185 unit of mass had disappeared and 17 mev of energy had appeared. To convert these figures into quantities we can use in the Einstein equation, we must remember that in atomic and nuclear physics the unit of mass is one-sixteenth of the mass of the predominant oxygen isotope, O^{16}, which is equal to

1.6603×10^{-24} gram. The unit of electric charge used is the positive charge of the proton, which is equal in magnitude but opposite in sign to the charge on the electron, and is often called the electronic charge; its value is 1.60×10^{-19} coulomb. The energy unit used in nuclear physics is the electron-volt, defined as equal to the kinetic energy which a particle carrying one electronic charge will acquire in falling freely through a potential drop of one volt. Since this is so tiny, the million electron volt unit (mev) is commonly used.

Returning now to the experimental results, 0.0185 mass unit is 3.07×10^{-26} gram, 17 mev is 27.2×10^{-6} erg, and c (the velocity of light) is 3×10^{10} cm per second. On substituting these values in Einstein's equation, $E = mc^2$, we have

$$27.2 \times 10^{-6} \text{ erg} = 27.6 \times 10^{-6} \text{ erg}$$

which is quite a good approximation to the equality of both sides.

Source of the Sun's Radiant Energy

According to C. G. Abbott, the energy received by the earth from the sun is about 1.7 horsepower per square yard (1.47 kw per square mile). This indicates an emission by the sun's surface of 7.88×10^4 horsepower per square yard (6.79×10^4 kw per square meter), which is heat enough to melt 39.6 feet of ice or to vaporize 5.92 feet of water per minute. Together, all the planets receive less than 1/120th of one-millionth part of this tremendous energy, which the sun has been pouring out for at least several billion years. Apart from the fact that solar temperatures enormously exceed those of any known chemical reaction, a sphere of coal the size of the sun, burning in pure oxygen, would be entirely comsumed in about 6,000 years. The notion that meteoric matter, falling into the sun, is a material factor in maintaining its heat has long ago been dismissed, as has also the notion of Helmholtz that solar contraction can account for the maintenance of solar heat.

The discovery of radioactivity, however, opened the door to new vistas. Thus R. A. Sampson[14] stated: ". . . if the sources of energy within the atom can be drawn upon, there is here an incalculable source of heat which takes the cogency out of any calculation respecting the sources of maintaining the sun's radiation. An equivalent statement of the same conclusion may be put thus. Supposing a gaseous nebula is destined to condense into a sun: the elementary matter of which it is composed will develop in the process into our known terrestrial and solar elements, parting with energy as it does so."

In 1897 Lord Kelvin advanced views as to the age of the earth, based on Helmholtz' contraction theory.[15] Professor T. C. Chamberlin (Chicago) replied thus to Kelvin's statements:[16]

"What the internal constitution of the atoms may be is still an open question. It is not improbable that they are complex organizations and seats of enormous energies. Certainly, no careful chemist would affirm either that atoms are really elementary or that there may not be locked up in them energies of the first order of magnitude. No cautious chemist would probably venture to assert that the component atomolecules, to use a convenient phrase, may not have energies of rotation, revolution, position and be otherwise comparable in kind and proportion to those of a planetary system. Nor would he probably be prepared to affirm or deny that the extraordinary conditions which reside in the center of the sun may not set free a portion of this energy. The Helmholtzian theory takes no cognizance of latent and occluded energies of an atomic or ultra-atomic nature."

Professor H. A. Bethe of Cornell has advanced[17] a theory, now generally regarded as reasonable, that the heat radiated by the sun is mainly due to a series of nuclear transformations which may be indicated thus:

(1) ${}_6C^{12} + {}_1H^1 \longrightarrow {}_7N^{13}$
(2) ${}_7N^{13} \longrightarrow {}_6C^{13} + {}_1e^0$
(3) ${}_6C^{13} + {}_1H^1 \longrightarrow {}_7N^{14}$
(4) ${}_7N^{14} + {}_1H^1 \longrightarrow {}_8O^{15}$
(5) ${}_8O^{15} \longrightarrow {}_7N^{15} + {}_1e^0$
(6) ${}_7N^{15} + {}_1H^1 \longrightarrow {}_6C^{12} + {}_2He^4$

The final result of these changes is the transformation of hydrogen into helium and positrons (${}_1e^0$), with the release of about thirty million electron volts. We know nothing of what is happening within the body of the sun, but to maintain its high rate of radiation, it must be losing mass at the rate of about 4,000,000 tons per second. It is consoling to remember that since the total mass of the sun is estimated to be 2.2×10^{27} tons, it would require about a million years for the sun to lose one ten-millionth of its mass, provided that nothing fell into it.

So terrific is the development of solar energy, that apart from the gigantic craters which we call "sun-spots," huge bursts of incandescent matter are being continually ejected from the sun's surface at the rate of thousands of miles per hour, and some of these "prominences" jump half a million or more miles outward. During the total eclipse of 1919 (see Figure 2) there was seen a

gigantic prominence resembling an ant-eater, 350,000 miles from snout to tail. Before setting of the sun removed it from visibility, it rose about 475,000 miles above the sun's surface.[18]

FIGURE 2. The Ant-eater prominence (May 29, 1919). The whole length of this prominence was about 350,000 miles. (*Courtesy A. C. Crommelin, Greenwich Observatory*; from "The Stars in Their Courses" by J. Jeans, The Macmillan Co., N. Y.)

The following comparison between chemical and nuclear heat is of interest, the chemical example representing an oxy-hydrogen flame:

$$4 \text{ grams } H + 32 \text{ grams } O = 36 \text{ grams } H_2O + 136{,}000 \text{ calories}$$

$$1 \text{ gram } H + 7 \text{ grams } {}_3Li^7 \rightarrow 8 \text{ grams } {}_2He^4 + 5 \text{ billion calories.}$$

The "annihilation energy" of one electron is 0.5 mev, while that of one mass unit is 931 mev.

Origin of the Solar System and the Age of the Earth

The Planetesimal Hypothesis of Professors T. C. Chamberlin and F. R. Moulton early in this century advanced the view that some ten or twenty billion years ago the sun passed sufficiently close to another star to raise vast tides and to stimulate greatly the sun's internal activities, so that vast streams of matter were ejected, both toward and away from the visiting star. While most of this matter fell back into the sun, about one-seventh of one per cent of the sun's mass remained swirling about the sun, all in one direction. The larger masses served as nuclei upon which the smaller ones collected into planets and their satellites. (Despite its nine moons, Saturn still has two rings, apparently consisting of unaggregated particles.) This hypothesis accounts well for the fact that all the planets and most of their satellites move in the same direction and approximately in the plane of the ecliptic. The few retrograde bodies may represent "captures," *e.g.*, of some cometary body.

Professor Lyman Spitzer, Jr.,[19] has attacked this hypothesis, and holds that material ejected by the sun or by a star would be too hot to condense to form separate bodies, but would dissipate into space.[20] Astrophysicists face conditions not found on our earth. Thus in some stars, for example, the "dark" companion of Sirius, atomic fragments are so closely packed that a single quart has an estimated mass of about 40 tons. On the other hand, atomic nuclei are much denser, as appears from the statement of Professor W. D. Harkins, quoted above.

As to the probable age of the earth, there is more agreement. According to Professor Louis B. Slichter,[21] geophysicists recognize three major divisions of the earth: (1) a heavy *core* with a mean density of 10.7, apparently fluid and possibly consisting of a mixture of nickel and iron; (2) a thick but much lighter *mantle* (density, 3.4 to 5.7) overlying the core and composed of ultrabasic rocks, the iron content increasing with depth; (3) the thin *crust* above the mantle (total thickness about 20 to 60 kilometers), consisting of several layers of lighter rocks. "It has long been recognized that the radioactive heat being generated in rocks is large enough to be a major influence in the earth's thermal history . . . Radioactive determinations of old crustal rocks indicate that the time which has elapsed since the solidification of the oldest rocks is about 1½ to 2 billion years."[22]

While some helium is probably lost, determinations on rocks preceding the Cambrian indicate an age for the solid earth of about 1,830 million years, and this checks fairly well with a "lead" age calculation as determined on uraninite found in Russia.[23]

Volcanism and Nuclear Energy

At Parícutin (Mexico) we have been witnessing the birth and formation of a volcano in what had been flat farming country. After three years the cone has reached a height of over three thousand feet, and in the fall of 1946 two new craters were reported emitting floods of lava. Professor Wilbur A. Nelson (Virginia) estimated that during the Cretaceous period a Tennessee volcano spat up over fifty cubic miles of material, part of which settled down to form beds of bentonite clay extending nearly 500 miles north and south, and nearly 400 miles east and west. But the Parícutin manifestations are trivial compared with what happened at Krakatoa within the memory of many now living.

Krakatoa is a small volcanic island in Sunda Strait between Java and Sumatra. A prehistoric explosion had blown away some old volcano, leaving an outer ring of islands outlining its huge crater. Later, the main cone, built up by subsequent eruptions, rose over 2,600 feet above sea level. About 1877 earthquakes began, and on Aug. 26th, 1883, came a series of gigantic explosions lasting three days, which blew away the entire north and lower portion of the island, leaving a bare vertical cliff which revealed the interior of the volcanic cone Rakata. It replaced heights of from 300 to 1,400 feet with submarine cavities 1,000 feet deep in some places. The huge amount of matter cast out to an estimated height of 17 miles gave off a colloidally dispersed dust cloud, or aerosol, which travelled to Europe, Asia, Africa, North and South America, and throughout Australasia. This cloud reached northern Scandanavia and the Cape of Good Hope, and caused brilliant sunsets for several years. Pumice floated for hundreds of miles, and ocean waves, some 50 feet high, reached Cape Horn (nearly 8,000 miles) and perhaps the English Channel (11,000 miles). More than 36,000 people were killed. The sound of the explosions carried nearly 3,000 miles, being heard in the Philippines, Ceylon, and in South and West Australia—by far the greatest distance sound has been known to travel. On the morning of Aug. 27th, a most powerful explosion originated an atmospheric wave which, being reflected or reproduced at the antipodes to

Krakatoa, "was observed not fewer than seven times at many of the stations, four passages having been those on the wave travelling from Krakatoa and three those of the wave travelling from its antipodes, after which its traces were lost" (Sir R. Strachy).

The comparatively recent eruption of Mont Pelé, Martinique, was much milder and more akin to the "atomic bomb." On April 25th, 1902, there was a discharge of ashes, and a heavy flow of lava occurred on May 2nd and 3rd. On May 8th a sudden explosion tore away the whole side of the mountain—the gash was clearly visible in 1939—and released what Zsigmondy called a *nuée ardente*—a cloud of superheated steam, gases, and colloidally dispersed particles of rock. About 40,000 persons perished, and all ships in the harbor, except the *Roddam*, were destroyed. Metal work on the *Roddam* was melted by the blast; but a prisoner in a dungeon cell in the town was later found alive, though spoons and glass goblets in the room above his cell had been melted. This bespeaks the intense but short-lived heat. It is known that under heat and pressure water "dissolves" in silicates, and the effects may have been due to the sudden explosive release of such a superheated mass, with atomization of the hot rock.

Dr. Leason H. Adams[24] has advanced the view that volcanism is based on radioactivity, and I give below a note of his theory which he kindly prepared at my request:

"The central problem of volcanism is to devise a mechanism which will explain the existence of local hot spots in the Earth's crust,[25] whereby at present, as during past geological epochs, eruptions of molten lava and hot gases may take place in localized areas. Volcanism is still a highly controversial subject among geologists and geophysicists, though many attempts have been made to explain it. A theory which has appealed to some of those who have given much attention to the subject attributes the local heating to slight variations in the average radioactive content of the rocks forming the crust in the active area. The theory is based on the following considerations:

"(1) The rocks of the Earth's crust contain a minute but very important amount of uranium and its disintegration products, including radium. In terms of radium, the amount of radioactive material in the usual igneous rocks is of the order of magnitude of one part in a million million; but the heat generated by even this minute amount of radium in a volume of rock measured in cubic miles, is very considerable.

"(2) The temperature gradient in the crust of the Earth (now judged to be more than 2,000,000,000 years old) is profoundly affected by

radioactive heat generated in the crust. Something like three-fourths of the present thermal gradient may be attributed to radioactive heat, and the balance to the residual heat of the originally molten globe.

"(3) Any reasonable interpretation of the laws of heat conduction leads to the conclusion that at moderate depths within the Earth's crust, say a few tens of miles, the temperatures are still very close to the melting points of the rocks at the particular depths.

"(4) From the foregoing it may be concluded that a relatively small increase in the radioactive content of the Earth's crust at a particular locality will raise significantly the temperatures of the rocks at depths miles below the surface, and may easily bring the temperatures above the melting point, thus maintaining a lava reservoir in that region. In other words, a somewhat greater amount of radium averaged through the vertical extent of the crust (but still very small in absolute magnitude), could account for the existence of a volcanic region.

"Against this theory, it has been urged that examinations of volcanic lavas and emanations have failed to reveal any striking increase in radioactive elements. It must be noted, however, that since the excess of radium required to produce a volcanic region is proportionally so small in amount, it would be difficult to establish experimentally, without careful sampling; and particularly that the examination of a few samples of rocks would not be conclusive as to the local average radioactive content in a particular region.

"It must be admitted that the explanation of volcanism on radioactive grounds has not been generally accepted, though some thoughtful geologists and geophysicists have been impressed with the idea. An alternative theory explains volcanism, in part at least, by chemical reactions taking place in the rising lava column. Perhaps the radioactive theory should best be regarded as a subject for further observation and analysis."[26]

The "Atomic Bomb"

The term "atomic bomb" is a *mumpsimus*—a Latin word introduced into the English language to indicate persistence in obvious error;* it originated in the fact that an old priest who had for forty or more years used this word, refused to change to *sumpsimus*, even when shown the correct word in the prayer book. The correct term is "nuclear fission" bomb.

It is fortunate for the world and for civilization that we were able to win the feverish race to produce the "atomic bomb"; for our enemies had been making desperate efforts to accomplish this difficult task and to use against us and our allies this most potent

* Abandonment of such an error is now termed a *sumpsimus*.

of all explosive weapons. We read the Smyth Report with mingled feelings—pride in the splendid scientific achievements revealed; joy for the speedy and enforced surrender of those who had set out to enslave the world, and for the saving of millions of lives on both sides—both soldiers and civilians—who would otherwise have perished had the war been continued; sorrow for the much lesser number who died in the nuclear explosions, and for the fact that these epoch-making scientific discoveries were prostituted to military uses, instead of being devoted to the benefit of humantiy.

It was already known that the isotope U^{235} would undergo nuclear fission by slow or thermal neutrons; but natural uranium contains only about 0.71 per cent of this isotope (AcU^{235}), 0.006 per cent of isotope $_{92}UII^{234}$, but 99.28 per cent of isotope $_{92}UI^{238}$. So besides finding out how to separate the isotope 235 for immediate use, a major problem was to find a way to utilize the major portion of the natural metal, by converting the U^{238} into something fissionable. Thomas Graham had developed the mathematical expression or "law" regarding gaseous diffusion, and though many other methods were investigated, the separation was successfully made by differential diffusion of uranium hexafluoride, UF_6. Since the rate of diffusion of gases through chemically indifferent septa is inversely proportional to the square roots of their molecular weights, the difference in the rate of diffusion between $U^{235}F_6$ and $U^{238}F_6$, whose molecular weights are 349 and 352 respectively, is very slight indeed. Immense technical difficulties had to be overcome in handling so corrosive and dangerous a substance during the 4000 diffusion cycles necessary.[27]

Meanwhile, it had been found that by radiating $_{92}U^{238}$ with neutrons, it could be transformed into $_{92}U^{239}$, by taking the neutron into its nucleus and emitting β-radiation. This new 239 isotope of uranium has a half-life of only 23 minutes; it emits an electron and becomes an entirely new element, neptunium, $_{93}Np^{239}$, which has a half-life of 2.3 days. Neptunium, in turn emits an electron and becomes another new element, plutonium $_{94}Pu^{239}$, which is relatively stable, having a half-life of 24,000 years, and emits alpha rays (helium nuclei).

Plutonium was "manufactured" in a "pile," consisting of a matrix of highly purified graphite with tubular passages into which were inserted aluminum "cans" containing uranium rods, protected from the cooling water needed to carry off the intense heat generated; for the production of 1 gram of plutonium per day involves liberation of energy at the rate of 500 to 1,500 kilo-

watts. The graphite matrix is needed to slow down high-speed neutrons sufficiently to obtain the desired fission effect. The intense radiation fields of the pile changed the electric resistance, the heat conductivity, and the elasticity of the graphite.

To maintain the pile in operation, conditions had to be chosen so as to maintain a "chain reaction" by continuous formation of sufficient neutrons, allowing always for the waste due to non-effective neutron collisions, absorption of neutrons by impurities, and escape of neutrons from the pile. Since the object of the pile is to produce plutonium by chain reaction, it is desirable to absorb all excess neutrons in U^{238}, leaving just enough neutrons available to maintain the chain by their action on U^{235}, aided by high-energy fission of U^{238} and thermal fission of Pu^{239}. While collisions between neutrons and U^{238} occur with neutrons of all energies, they are most probable with those whose energies lie in the "resonance" region, located somewhat above thermal energies. So great is the tendency of the dominant isotope U^{238} to absorb neutrons compared to their tendency to cause fission of the 140 times less numerous U^{235} atoms, that this fission had to be favored by use of materials of high purity, by a suitable lattice, use of a moderator, etc.

After sufficient plutonium had been produced in the pile, the slugs of uranium were withdrawn and dissolved, and the plutonium was separated by co-precipitation with some other element, an expedient common in radioactive chemistry. The chemistry of plutonium had been worked out previously, on an ultramicrochemical scale.

Because of the extremely dangerous radiations emitted throughout the several processes, they must all be operated by remote control, the personnel being shielded by heavy concrete and metal walls. Smyth states that plutonium is one of the most dangerous substances known if it gets into the body; and the fission products, which include some thirty elements, are also very active and troublesome. These major fragments of the fission of uranium are released in considerable quantity when the slugs of uranium are dissolved, and high stacks had to be built to carry off the deadly gases without endangering the surrounding countryside.

Most of the technical difficulties involved in assembling the "atomic bomb" arose from the fact that the time interval between the beginning and the end of a chain reaction is extremely short. The efficiency of the bomb depends on the ratio of (a) the speed with which neutrons emitted by the first fissions can get to other

nuclei and produce further fission, and (b) the speed with which the material of the bomb flies apart. Since a mass of plutonium or of U^{235} above a critical size would spontaneously undergo explosive fission, to produce satisfactory detonation the bomb must consist of separate pieces so small that they are incapable of doing this. These are shot together to create the explosion.

This "critical size factor" is one of the most amazing properties of fissionable materials. The "critical mass" necessary for explosion is guessed to be from five to fifteen pounds.

The Successive Levels of Material Structure

The accompanying table shows the various material units at present recognized, and their position in order of size and mass relative to units which are generally known. It must not be assumed, however, that our present inability to break up the "ultimate" units, *e.g.*, protons, electrons, etc. represented on the diagram as of zero order of complexity, is to be considered as final evidence of their actual simplicity. In fact, the "spin" of an electron is a factor well recognized in atomic and nuclear physics, and this may be due to some kind of complexity. Besides, we cannot, even in imagination, go down to the ultimate particle or particles of matter, even though we recognize the realities which emerge from them.

In order to locate man and biological happenings in their relative position in universe, especially introducing the essential time factor so often omitted from consideration, there is also given a table showing measurements in space, mass, and time, reaching in each case from the greatest to the smallest known or readily calculable "unit."

Dr. Edwin Hubble (Mt. Wilson Observatory, Carnegie Institution) compares our stellar system to a drifting swarm of bees, the sun, with its relatively insignificant family of planets, being a fairly small star in the swarm. Astronomers, peering out into the vast emptiness of space, see other stellar systems so distant that they appear merely as unresolvable patches of light, and are therefore called *nebulas.* The "observable region" is a sphere, roughly 600 million light-years in diameter, within which are scattered about 100 million nebulas, averaging 1½ million light-years apart, 80 million times brighter than the sun, and 800 million times greater in mass. The faintest nebulas that our present telescopes can detect lie at the peripheral surface of this sphere. Whatever

SUCCESSIVE LEVELS OF MATERIAL STRUCTURE

Elementary Particles

Order of Complexity 0 (?)	Charge	Size (Å)	Mass (gram)	Discoverer	Where Found
Electron	$-e$	3×10^{-5}	9×10^{-28}	Sir J. J. Thomson (G. Johnstone Stoney) (Sir Wm. Crookes)	Electric current Negative electrostatic charges Nuclear satellites and emissions
Positron	$+e$	3×10^{-5}	9×10^{-28}	C. D. Anderson	Emitted by atomic nuclei
Proton	$+e$	2×10^{-8}	1.66×10^{-24}	Sir Ernest Rutherford	Nucleus of protium (H)
Neutron	0	2×10^{-5}		James Chadwick	Atomic nuclei
Positive Meson	$+e$	2×10^{-5}	2×10^{-25}	C. D. Anderson and S. Neddermeyer	Cosmic radiation
Negative Meson	$-e$	2×10^{-5}	2×10^{-25}	C. D. Anderson and S. Neddermeyer	Cosmic radiation

QUANTA OF INTERACTION now recognized as providing distinct means for the transfer of energy between one elementary particle and another:

Photon: Elementary unit of radiation energy carried away by the electromagnetic field from a radiating atom or other system of particles.

Gravitron: Elementary unit of gravitational energy carried off by way of gravitational forces from an oscillating system of particles.

Neutrino: Elementary unit of energy given off by a radioactive nucleus in every decay process in which simultaneously an electron or a positron is released.

Successive Levels of Material Structure (*Continued*)

Material Structures	Order of Complexity	Mode of Examination	Approximate Size	*BIOLOGY AND MEDICINE* Chemical Units	Biological Units	*TECHNOLOGY* Cotton	Starch	Carbon Steel
Polyelectrons*........	1							
Atomic Nuclei.......	2	Transmutation Cyclotron	He = 8×10^{-5} Å Au = 1×10^{-4} Å					
Atoms	3	Cyclotron X-Ray Chemical	5 Å ±	C, H, O, N, P, S, Fe, K, Na, Ca, Mg, Cl, Fl, B, I, Zn, Cu, Cr, Ni, V		C, H, O	C, H, O	Fe, C(0.1 – 1.0%) (S, P, Si, Mn, etc.) 0.5% C = ca 7.5% FeC_4
Molecules...........	4	Infrared and Raman Spectra, X-Ray, Chemical	50 Å ±	Amino acids, Lipoids, Nucleotides, Glucose, etc.	Vitamins, Hormones, Thiamin Pyrophosphate, Carriers ‡, etc.	Glucose, etc.	Glucose	Fe, C, FeC_4
Macromolecules†..... Molecular Aggregates.	5	Electron Microscope Ultramicroscope Chemical	100 mμ ±	Proteins, Glycogen, Cellulose, Starch, etc.	Enzymes, Chlorophyll, Cytochrome, Bacteriophages, Genes, Viruses, etc.	Molecular Chains and Groups	Molecular Chains and Groups	α-iron (Ferrite) γ-iron Cementite
Micells..............	6	Electron Microscope Ultramicroscope	1 μ ±		Cytoplasmic structures	Aggregates with impurities		
Microscopically Resolvable Units.....	7	Chemical Microscope	¼ μ ±		Chromosomes, Nuclei Cells, Bacteria	Fibrils	Starch grains	Austenite (α-iron) Martensite (γ-iron) Troostite Sorbite Pearlite
Visually Resolvable Units...............	8	Eye	50 μ ±		Tissues, Organs, Drosophila, Mouse, Whale	Fibers, Yarns Fabrics, Clothing		Hair-spring, Saw, Engine, Bridge, Sky-scraper

† Primary Colloidal Particles................. 30 mμ ±
Secondary Colloidal Particles.............. 100 mμ ±

‡ Moleculobiont, hypothetical simplest living unit.

* A polyelectron is a cluster of electrons and positrons held together by their mutual electrical interactions in a system having properties (apart from the absence of a nucleus), similar to those of atoms. See paper by John A. Wheeler, *Ann. N. Y. Acad. Sci.* (1946) 48, 219-238.

Space	*Mass*	*Time*
Meters	Grams	Seconds
1.9×10^{26} Einsteinian Universe	8×10^{78} Universe	1.5×10^{19} Sun to Lose ½ Mass
4.7×10^{20} Milky Way, Diameter	3×10^{55} Milky Way	6×10^{16} Age of Earth
		Eras — Million Years
		Archaeozoic — ca 1,000
		Proterozoic — 500
3.2×10^{16} Parsec { Distance of a Star Whose Parallax = 1 Second of Arc		2.6×10^{16} { Paleozoic — 300
		Mesozoic — 125
		Cenozoic — 75
9.5×10^{15} Light-year—ca. 9½ Million Million km		
1×10^{13} Solar System—Dia. 10 Billion km		6×10^{13} Quaternary Period, Man Appears
1.5×10^{11} Earth to Sun—149 Million km		2.2×10^{11} Recorded History 7,000 Yrs.
6.9×10^{8} Sun-Radius—692,000 km	2×10^{33} Sun	2.3×10^{6} Lunar Month—27.8 Days
6.4×10^{6} Earth-Radius—6377 km	5.99×10^{26} Earth	8.6×10^{4} Mean Solar Day
1.7×10^{0} Man—Average Height 1.7 m	7×10^{4} Man 166 Lbs.	ca. 9×10^{0} 100 Yard Dash
ca. 5×10^{-6} Cells, Drosophila { Nucleus 5-10μ	4×10^{-10} Sensitivity of Microbalance	1×10^{-3} Motor Nerve Impulse
Chromosomes 0.2-3μ	0.4γ	6.4×10^{-2} Motion Picture on Screen 64σ
8×10^{-6} Red Blood Corpuscle—7,500 mμ		
2×10^{-7} Coccus—ca. 0.2μ	2×10^{-12} Mercaptan Odor Limit	
	0.002γ	
2.5×10^{-7} Limit of Microscopic Resolution		1×10^{-6} Oscillograph
1×10^{-7} Large Colloid Particles		8×10^{-8} Radio Oscillation 80$\mu\sigma$
1.3×10^{-8} Shortest Ultraviolet—13mμ	ca. 2×10^{-18} Colloids	
5×10^{-9} Small Colloid Particles—Oil Films		
$0.2-5 \times 10^{-9}$ Molecules—0.2-5mμ	3×10^{-23} Water Molecule	
$1-6 \times 10^{-10}$ Atoms—100-600$\mu\mu$	1.66×10^{-24} H-Atom	
1.3×10^{-11} Electron Orbit—H-Atom 53$\mu\mu$	9×10^{-28} Electron	
1.9×10^{-11} Hard X-Rays—19$\mu\mu$		5×10^{-14} Yellow Light Oscillation
5×10^{-14} Cosmic Rays—50 Rutherford Units		
3.8×10^{-15} Electron—Dia. 3.8×10^{-5}A		
1×10^{-15} Rutherford Unit 0.00001A		
2×10^{-18} Proton—H-Nucleus 2×10^{-7}A	1.66×10^{-24} Proton	1×10^{-22} Cosmic ray Oscillation
1km = 1,000m 1m = 1,000mm 1mm = 1,000μ	1g = 1,000mg 1mg = 1,000μg	1 Second = 1,000σ 1σ = 1,000mσ
1μ = 1,000mμ 1mμ = 1,000$\mu\mu$ 1mμ = 10A	1μg = 1,000γ 1γ = 0.000,000,001g	1mσ = 1,000$\mu\sigma$

matter may be scattered in space between the nebulas does not dim appreciably the most distant ones now visible. Dropping this from consideration, the concentration of matter in the visible region figures out to about one gram per 10^{30} cubic centimeters, which is equivalent to one grain of sand in each volume of space equal to that of the earth.[28]

Plummeting down to our own tiny but (to us) important levels, we find that life depends upon a great variety of ordered chemical reactions which are rather limited in space, mass, and time. Living units have masses ranging from the molecular or near-molecular up to the immense bulk of the whale or elephant. The chief chemical elements involved are carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, sodium, potassium, calcium, iron, magnesium, and manganese; but quite a number of other elements are also essential or incidental, in amounts small in percentage but large in numbers of atoms, *e.g.*, copper, zinc, fluorine, vanadium and molybdenum. From this quintessence of dust, living units have emerged on our tiny planet which in the slow course of time developed brains and bodies with which they can receive and order sense impressions, remember, think, and reason. How all this happens, no one knows; but the result is undoubted, irrespective of what *words* are used to describe it—mind, psyche, soul, etc. It is recognized in our laws, is studied by psychologists, psychiatrists, sociologists, and teachers, and dominates the daily life of every one of us.

In a biographical sketch of Emil Dubois-Reymond, apparently written by Sir Michael Foster,[29] the following is quoted as the dominant note of "Elements of Physiology" by Johannes Müller, central figure of modern physiology:

"Though there appears to be something in the phenomena of living beings which cannot be explained by ordinary mechanical, physical or chemical laws, much may be so explained, and we may without fear push these explanations as far as we can, so long as we keep to the solid ground of observation and experiment."

Since we now have a continuity of successive levels of material structure down to the nuclear level, there is no longer any "no-man's land" where material mysteries of life can escape scientific investigation. Our present knowledge, based on observation and experiment, indicates that the simplest living units are in the molecular or near-molecular range. The information assembled in this book indicates that many, if not all the basic material facts

of life are understandable on catalytic principles, including selective adsorption and differential diffusion. But the mental and spiritual phenomena which emerge, and which are just as real as the material ones, are as inscrutable as ever.

REFERENCES

[1] For further historical aspects, reference must be made to books on the history of science, *e.g.*, that of Sir William C. Dampier (3rd ed., Macmillan Co., New York, 1944).

[2] Stoney's original papers are in Proc. Brit. Assoc. Adv. Science, Belfast meeting, 1874, and in *Trans. Roy. Dublin Soc.*, (1891), 4, 583. See also "The Electron Theory" by Fournier d'Albe, London, 1907.

[3] *Comptes rendus*, July, 1898, "On a New Radioactive Substance Contained in Pitchblende."

[4] English translation by Victor Sheean, Doubleday, Doran & Co., Inc., 1937.

[5] "On the Occurrence of Nitrogen in Uraninite and on the Composition of Uraninite in General," U. S. Geol. Bull., 78, 43-79 (1889-90).

[5a] About 1865 the great Belgian chemist Jean Servais Stas wrote: "I have arrived at the absolute conviction, the complete certainty, so far as is possible for a human being to attain to certainty in such matters, that the law of Prout is nothing but an illusion, a mere speculation definitely contradicted by experience." (It should be stated, however, that shortly before his death in 1891, on noting the close approximation to integers shown by a number of atomic weights when hydrogen is taken as unity, Stas remarked: "Il faut croire qu'il y a quelque chose la-dessous.") But in 1932 Soddy wrote: "After many vicissitudes and the most convincing apparent disproofs, the hypothesis thrown out so lightly in 1815 by Prout, an Edinburgh physician, has a century later become the corner-stone of modern theories of the structure of atoms. There is something surely akin to, if not transcending tragedy in the fate that has overtaken the life work of that distinguished galaxy of nineteenth century chemists, rightly revered by their contemporaries as representing the crown and perfection of accurate scientific measurement. Their hard-won results, for the moment at least, appear as of little interest and significance as the determination of the average weight of a collection of bottles, some of them full and some of them more or less empty."

[6] Isotopes of tin:

Atomic Wt.		*Percentage*
112	—	1.1
114	—	0.8
115	—	0.4
116	—	15.5
117	—	9.1
118	—	22.5
119	—	9.8
120	—	28.5
122	—	5.5
124	—	6.8

[7] In his book "Classical and Modern Physics" p. 564, New York, D. Van Nostrand Co., 1940.

[8] See his extensive historical review in *Science*, 1946, 103, 289-302.

[9] The reader must consult special books and journal articles regarding these matters, and also about mesons, cosmic rays, and other developments.

[10] *Science*, Oct. 25th, 1946.

[11] See *Physical Review*, Jan. 15th and Feb. 15th, 1939.

[12] "A General Account of the Development of Methods of Using Atomic Energy for Military Purposes," (Princeton Univ. Press, 1945).

[12a] Lorentz, equation is

$$m = \frac{m_0}{1 - \left(\frac{v}{c}\right)^2}$$

where m is the mass of the moving electron, m_0 its mass when at rest, v its velocity, and c the velocity of light in a vacuum.

[13] In *Nature*, Nov. 1913, F. Soddy suggested that while the principle of relativity indicates that relative velocities greater than that of light are physically impossible, yet there is an actual possibility of observing in nature a relative velocity considerably greater. Thus if two β-particles are shot out by a radioactive body in opposite directions, each with a velocity nine-tenths that of light, their actual relative velocities would be $1.8c$. Regarding this E. Cunningham ("Relativity and the Electron Theory," Longmans, Green & Co., London, 1915) states (p. 40): "This is quite true, and the principle of relativity has nothing to say against it. The principle maintains that a velocity greater than c *relative to the observer* cannot be observed. . . . The position is not that velocities greater than c are not conceivable, but that real bodies become illusory in observation if they are conceived to be moving faster than light. We shall also see later that the electrical constitution of matter seems to indicate that a body would suffer dissolution if it were accelerated so that its velocity were made greater than c."

[14] Enc. Brit., 11th ed., 1910.

[15] One of his addresses on this topic appeared in *Science*, 1899, **9**, 666-74, 704-11.

[16] *Science*, 1899, **9**, 889-901, and **10**, 11-18.

[17] *Astrophysical Jour.*, 1940, **92**, 118.

[18] The late Dr. W. S. Andrews (General Electric Co.) published in *Scientific Monthly*, Dec., 1928, **27**, pp. 535-8, a paper entitled: "Hypon—A Hypothetical Element and a Possible Source of Stellar Energy," in which he suggested the possibility that what astronomers call *novae* are due to the sudden and terrific explosion of "hypon," mass 118. He gave the following table, showing the position of this hypothetical "element," which we would now speak of as "transuranic":

Helium	$2(1^2)$	2
Neon	$2(1^2+2^2)$	10
Argon	$2(1^2+2^2+2^2)$	18
Krypton	$2(1^2+2^2+2^2+3^2)$	36
Xenon	$2(1^2+2^2+2^2+3^2+3^2)$	54
Radon	$2(1^2+2^2+2^2+3^2+3^2+4^2)$	86
"Hypon"	$2(1^2+2^2+2^2+3^2+3^2+4^2+4^2)$	118

[19] *Astrophysical Jour.*, 1939, **90**, 675-88.

[20] C. S. von Weizsaker (*Astrophysical Journal*, March, 1945) proposed a different hypothesis, and still others are discussed in "The Observatory" for August, 1945. Evidently there is no agreement as to the mode of origin of the solar system.

[21] Bull. Geological Soc. of America, April 1, 1941, pp. 562-99.

[22] Since $_{92}UI^{238}$, $_{92}AcU^{235}$, and $_{90}Th^{232}$ are continuously degenerating at constant speed into $_{82}Pb^{206}$, $_{82}Pb^{207}$, and $_{82}Pb^{208}$, with the respective liberation of 8, 7, and 6 helium nuclei, geophysicists use an "age equation" of the following form:

$$\text{Age} = \frac{\text{Amount of disintegration product}}{\text{Rate of production of disintegration product}}$$

"Age" means the time elapsed since the final solidification of the rock or mineral containing the radioactive element.

[23] For further details, see a paper by Professors Clark Goodman and Robley D. Evans (M.I.T.), Bull. Geol. Soc. of America, April 1st, 1941, pp. 492-541.

[24] *J. Wash. Acad. Sci.*, 1924, 14, 459-472; *Trans. Am. Geophys. Union*, 1930, pp. 309-41.

[25] The Byrd expedition reported (1947) the presence of two "oases" in Antarctica. Coal is mined in Spitzbergen, only ten degrees from the North Pole. About 1½ billion years ago radioactivity from U^{235} alone was four times what it is at present. J. A.

[26] See also A. Knopf, in "A Textbook of Geology," by Longwell, Knopf and Flint, Part I, 2nd ed., J. Wiley & Sons, Inc., New York, 1939.

[27] For further details, see W. D. Harkins, *Science*, March 8th, 1946, p. 293, and Smyth Report, 9.14.

[28] *Scientific Monthly*, Sept., 1934.

[29] Encylo. Brit., 11th ed., 1910, 8, 626.

Chapter 3

How Molecules Make Masses

Atoms and molecules are so tiny that they seem quite remote. Only under unusual experimental conditions do we deal with the effects which they produce as individual bodies of matter. In the practical affairs of life and in most scientific investigations as well we are concerned with large numbers of atoms or molecules which, when gathered together, form masses large enough to be experimented upon, or even to become microscopically visible. The smallest particle we can distinguish or "resolve" in microscopes may contain many millions of the smaller molecules, or a great number of macromolecules.

The convenient shorthand used by chemists to describe a compound, generally called its *formula* (little or concise form) is far removed from what that molecule may actually be like under natural conditions. When Ira Remsen of Johns Hopkins University presented his students with the customary formula for a double salt, *e.g.*, $PtCl_4 . 2KCl$, he would point to the period in the formula and remark with a twinkle in his eye, "That period has for many years been a full stop to thought. Don't let such devices keep you from trying to find out what lies behind them." Even the more informative "structural formulas" are only static diagrams, which give no indication of the dynamics of atomic and molecular structure, though in some cases straight or curved arrows are added to indicate oscillatory or resonance changes.[1]

Though three-dimensional isomers (stereoisomers) have been known since the discovery by Pasteur of right- and left-handed tartaric acids, the extent of this phenomenon and its biological importance are only now being increasingly recognized. For example, Professor L. Zechmeister of the California Institute of Technology[1] gives skeleton models of the twenty stereoisomers of beta carotene. (See Figure 3). Many of the naturally occurring carotenoids have a much larger number of calculable stereoisomers, two having as many as 128.

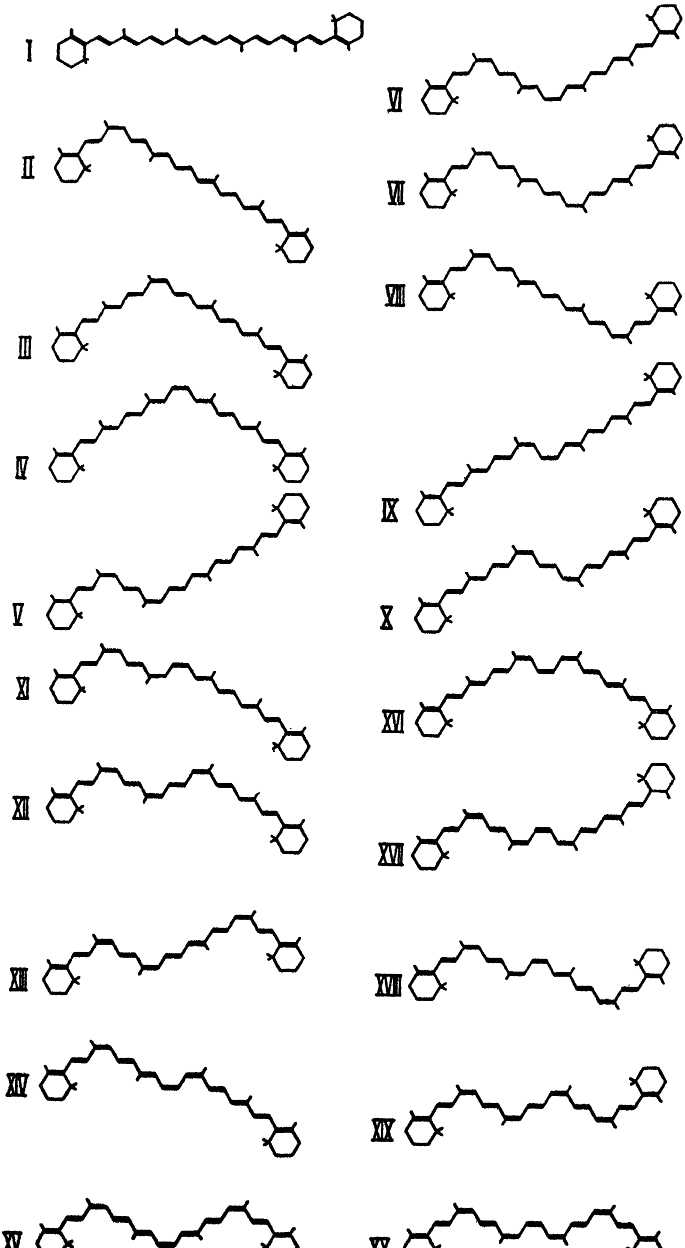

Figure 3. Skeleton models of the twenty possible stereoisomers of β-carotene: all-*trans*-β-carotene, three mono-*cis*-β-carotenes, six di-*cis*-β-carotenes, six tri-*cis*-β-carotenes, three tetra-*cis*-β-carotenes, and all-*cis*-β-carotene. (*Courtesy Chemical Reviews*)

Crystals and Colloids

Water is commonly referred to as H_2O, and many know that there are also present a small number of molecules of "heavy water" (deuterium oxide, D_2O), the average ratio being one molecule of deuterium to 6,500 of hydrogen. The physical chemist, however, is aware that under ordinary conditions water molecules undergo *dissociation* into H^+ and OH^-, with recombination at such a rate that with very pure water at 22° C we have at any instant a concentration of these oppositely charged ions of 1/10,000,000 mole per liter, which equals $\frac{1}{10^7}$ or 10^{-7} mole per liter. This is commonly expressed by saying that pure water has a pH of 7, which means that its concentration of hydrogen ions is expressed by the mathematical exponent 7, deprived of its minus sign. The French expression first used by Sørensen was *"pouvior hydrogene,"* or hydrogen power (in the mathematical sense of the word "power"). As a consequence, the intervals on the pH scale, being exponential, are vastly wider than the figures themselves might indicate. This may be seen from the following table:

	pH	Number of times H^+ or OH^- concentration exceeds that of pure water at 22° C
	1	1,000,000
	2	100,000
Acid side	3	10,000
(excess of H^+ ions)	4	1,000
	5	100
	6	10
Neutrality →	7	0
	8	10
	9	100
Alkaline side	10	1,000
(excess of OH^- ions)	11	10,000
	12	100,000
	13	1,000,000

In most living units the pH hovers near neutrality, the pH of the blood in man ranging from 7.30 to 7.45, the former approaching "acidosis" which is really a dimished alkalinity. The cytoplasm of immature starfish eggs shows a pH of about 6.6 to 6.8, while that of the nucleus is 7.6 to 7.8. However, the skin is usually quite acid (about pH 4.2 to 5.6) and Professor Frank C. Combes states[2] that this acid mantle is an important factor in the protection of the organism against invasion by bacteria and fungi.

In certain protected spots, (*e.g.*, the axilla and between the smaller

toes), the pH may be closer to alkalinity and become favorable to the growth of some micro-organisms. Thus, the fungus *Tinea,* which causes "athlete's foot" commonly grows between the toes, and may be combatted by acid germicidal ointments containing, *e.g.*, salicylic acid and sulfur; and the highly acid sulfate of alumina is the basis of most cosmetics used to combat under-arm odor. So old is this custom that the elder Pliny (Gaius Plinius Secundus), the Roman encyclopedist who perished while investigating the great eruption of Vesuvius which buried Pompeii and Herculanium in 79 A.D., mentions in his "Natural History"[3] that "alum is used for offensive odors in the axilla." In the stomach the gastric juice has an acidity of between pH2 and 3, corresponding to about 0.1 to 0.2 per cent hydrochloric acid. In some snails and ascidians (*e.g., Phallusia*), the blood may be quite acid.

Water has one other peculiarity which is generally overlooked, namely, the tendency of its molecules to form groups, probably in part through hydrogen bonds. This phenomenon, common to many other substances[4] is known as *molecular association;* and water contains double and triple molecules (dihydrol and trihydrol) whose percentages vary with conditions. According to N.E. Dorsey,[5] many accept the quasi-crystalline theory of water structure sponsored by J. D. Bernal and R. H. Fowler[6] and M. L. Huggins,[7] which demands a fairly rigid structure for water. But it must be remembered that, apart from whatever structure water may assume as the result of a kinetic equilibrium of its ions and molecules, a supermolecular structure may at times appear at levels higher than those before mentioned. Thus Professor H. T. Barnes of McGill University, in speaking of *frazil*—a type of ice occasionally giving much trouble to users of hydraulic power by obstructing the flow of water like the slimy precipitates met with in chemical analysis—says that when first formed it is too fine to be seen by the eye, apart from its action on the color of the water. But it soon agglomerates into spongy masses of loose texture, which readily catch onto subjects in the water, and quickly build large masses. The slightest drop in temperature below 0° C may cause frazil to appear. "The balance is so delicate that it will escape detection by the most sensitive thermometer and the first indication that a drop has occurred will be the appearance of ice." The action of frazil on the appearance of the water brings to mind the familiar glooming or darkening of clouds when rain impends.

It is pertinent here to quote the remarks of Thomas Graham (1861): "Ice itself presents colloidal characters at or near its melting point,

paradoxical though the statement may appear. When ice is formed at temperatures a few degrees under 0° C, it has a well-marked crystalline structure, as is seen in water frozen from a state of vapor in the form of flakes of snow and hoar-frost, or in water frozen from dilute sulfuric acid, as observed by Mr. Faraday. But ice formed on contact with water at 0° C is a plain, homogeneous mass with a vitreous fracture, exhibiting no facets or angles. This must appear singular when it is considered how favorable to crystallation are the circumstances in which a sheet of ice is slowly produced in the freezing of a lake or river. The continued extraction of latent heat by ice as it is cooled a few degrees below 0° C, observed by Mr. Persons, appears also to indicate a molecular change subsequent to the first freezing.

"Further, ice, although exhibiting none of the viscous softness of pitch, has the elasticity and tendency to rend seen in colloids. In the properties last mentioned it suggests a distant analogy to gum incompletely dried, to glue or any other firm jelly.

"Ice further appears to be of the class of adhesive colloids. The reintegration (regelation of Faraday) of masses of melting ice when placed in contact has much of a colloidal character. The colloidal view of the plasticity of ice demonstrated in glacial movement will readily develop itself.

"A similar extreme departure from the normal appears to be presented by a colloid holding so high a place in its class as albumen. In the so-called blood-crystals of Funke, a soft gelatinous, albumenoid body is seen to assume a crystalline contour.

"Can any facts more strikingly illustrate the maxim that in nature there are no abrupt transitions, and that distinctions of class are never absolute?"

It is of interest to note that the word "crystal" arose from the notion that rock crystal (clear, transparent quartz obtained from the Alps in ancient times), had been formed from water by intense cold. The word is derived from the Latin *crystallum* (clear ice) or the Greek *krystallos,* (from *kryos,* frost). Not until the 17th century was the word extended to the crystals familiar to all of us. In fact, the Romans called many crystals *glasses* (*vitrum*), and this survives in such expressions as *blue vitriol* (copper sulfate crystals), *white vitriol* (zinc sulfate), and *green vitriol* (ferrous sulfate). The corrosive, oily liquid obtained by distilling "green vitriol" was called "oil of vitriol."

While there is no space here to consider the experimental and theoretical aspects of crystals and crystallization, a few popular misconceptions should be set straight. First, Thomas Graham, the father of colloid chemistry, was well aware of the fact that the same substance may exist either in the colloidal or the crystalloidal

state. He wrote:[8] "For the mineral forms of silicic acid, deposited from water, such as flint, are often found to have passed, during the geological ages of their existence, from the vitreous or colloidal into the crystalline condition (H. Rose). The colloidal is, in fact, a dynamical state, the crystalloidal being the statical condition."

We now know that the basis of colloidality is degree of dispersion (size or dimension of particulate units), and that many colloidal or "amorphous" substances contain crystals or colloidal dimensions, though non-crystalline aggregates may also be present. In the early part of this century, P. P. von Weimarn showed that any substance may be obtained either in the colloidal or the crystalline state, depending on conditions of its formation.[9] Recently, rubber hydrocarbons and viruses have been crystallized. The devitrification of glass by slow crystallization may make it turbid and brittle, a condition sometimes noticed in old chemical glassware and tubing. Professor Alexander Silverman of the University of Pittsburgh kindly gave me a well-developed crystalline globulite obtained from a tank of glass that had cooled slowly when a factory suddenly shut down and was later abandoned. I have seen a similar globulite said to have been found in an ancient Mesopotamian glass furnace.

What may happen, if we wait long enough for results, is also shown by the huge deposit of travertine (a calcareous rock), at Mammoth Hot Springs, Yellowstone National Park, Wyoming. Owing to the sudden release from solution and loss of carbon dioxide, and assisted by the crystal-inhibiting action of the colloidal algae which grow on the overflow surfaces, the freshly deposited travertine has a filamentous or or cryptocrystalline structure. The older deposits show increasing evidence of crystallinity, and in the oldest deposits at the top of the terrace-hill, estimated by geologists to have been laid down twenty to thirty thousand years ago, good-sized, sparkling crystals are found, which still maintain the rhythmic rings of the original deposit.

Graham had remarked: "The formation of quartz crystals at low temperature, of so frequent occurrence in nature, remains still a mystery. I can only imagine that such crystals are formed at an inconceivably slow rate, and from solutions of silicic acid which are extremely dilute. Dilution no doubt weakens the colloidal character of substances, and may therefore allow their crystallizing tendency to gain ground and develop itself, particularly where the crystal, once formed, is completely insoluble, as with quartz." We must consider, however, another mode of crystal formation, which,

though it may not be true of quartz, should clarify the mystery Graham refers to.

Petrographers have long noticed the incipient crystal forms (crystallites) which appear in media where high viscosity and quick cooling oppose crystallization, as in obsidian, pitchstone, etc. Such forms can be obtained artificially from a solution of sulfur in carbon disulfide, made viscous by the addition of some Canada balsam. If a drop of this solution is rubbed out on a microscope slide and blown upon to speed the evaporation of the solvent, any or all of the following stages may be seen on microscopic examination: (1) tiny globules (possibly containing ultramicroscopic crystals); (2) aggregations of these globules into bead-like strings or margarites (Greek, *margaritis,* a pearl), or into geometrical groups; and (3) tiny crystals surrounded by empty spaces or lacunae, indicating that the surrounding material had gone to make up the crystal.[10]

The various intermediate steps in the formation of visible crystals are very often observable in window-pane ice, when conditions are right. In fact, during a half-hour ride in a Fifth Avenue bus in New York City on a very cold day, the transition from the "ground-glass" effect of globules, to dendrites (tree or fern-like formations), and ice-crystals with lacunae, took place before my eyes. Mr. Wilson A. Bentley of Jericho, Vermont, had for years made photographs of a great many varieties of the frozen forms of water, including snow, hoar-frost, hail, and window-pane ice. On seeing some of these printed in the *National Geographic Magazine* of 1923, and later in a copy of the *Monthly Weather Review* for Nov. 1907, which Mr. Bentley kindly sent me, I recognized each of the phenomena above mentioned. Besides, there were a number of cases where specific dendritic forms indicated the presence on the glass of sodium chloride and some protective colloid, both perhaps coming from the soap used to clean the window-pane.

The usual course of crystallization seems to proceed in the following steps: (1) as drop in temperature or loss of solvent lessens the capacity of the magma or solvent mass to hold the atoms, ions, or molecules of the dispersed substance in true solution, they form groups which clump together into tiny crystals or amorphous heaps (*crystallogens*); (2) when these clumps become large enough, their kinetic activity drops, and they are aggregated by surface forces into spherulites; (3) the spherulites then unite to form

dendritic or larger groups; (4) the tiny units now slip, snap, or reorient to form large crystals, which are usually full of tiny imperfections, as many, including Sir William H. Bragg, Smekal, and Zwicky have noted.

According to Professor G. W. Stewart[11] x-ray and other evidence indicate that the molecules within a liquid fall roughly into two classes: (1) those comparatively free; (2) those in semi-orderly array. He terms these fluctuating molecular "mobs" *cybotactic groups*, which in the case of *liquid* para-azoxyanisol, may consist of perhaps 100 to 1,000 molecules. In a *liquid crystal* of this substance these companies are ordered into regiments composed of perhaps millions of molecules. In the case of numerous kinds of liquid crystals (O. Lehmann, 1889), these "regiments" form still larger groups of various kinds, some *smectic* (soap-like), others *nematic* (worm-like).[12]

In 1907, I independently observed the effects of colloids on crystallization. But the phenomenon has been repeatedly noted by others.[13] Many artifacts, bearing remarkable resemblances to structures developed by living units, have been produced by formation in the presence of colloids.[14] In 1925 I drew special attention[15] to a most remarkable but unappreciated book by Dr. William M. Ord.[16] This book, which I first chanced to see advertised in a second-hand dealer's catalog, sums up the work done by Ord, George Rainey of St. Thomas' Hospital, Professor Hartung of Utrecht, and others, much of which is buried in *oubliette* journals. Of the many interesting observations (*e.g.*, the use of double colloidal protection) I here give the following excerpt:[17]

"It will be useful first to review the more important of the processes by which the modification of the crystalloid to the spheroidal form is effected.

"(1) A new salt is formed by decomposition, and caused to combine in its nascent state with the colloid . . . The colloid may be of the proteid or the gelatinous kind, or amyloid, or pectous, or an isomeric modification of an organic crystalloid, as uric acid, or of an inorganic crystalloid, as silicic acid, peroxide of iron, etc. Thus Mr. Rainey mixes two gummy solutions of the reacting salts;* Professor Hartung places solid salts at different points of an albuminous solution; in my own plugged tubes the reacting solutions are placed on opposite sides of a thick colloid dialyzer, within which they meet and undergo mutual decomposition. A modification of this condition is employed when urate of soda is formed by boiling uric acid with strong solutions

* This is actually double colloidal protection. J. A.

of chloride of sodium or phosphate of sodium. The urate comes down in a colloidal state, and affects included crystals as any other colloid would.

"(2) A crystalloid is deposited from solution in the presence of a colloid. This occurs in Dr. Guthrie's experiment with sulfate of copper and gelatin, in uric acid and urates mixed with albuminous urine.

"(3) Crystals are broken down and turned into spheres or spheroids or molecules, by the action of colloids into which they are introduced—a condition constantly seen in microscopical preparations. Crystals of uric acid, carbonate or oxalate of lime, etc., when preserved in gelatin or glycerine or glycerine-jelly, speedily lose their transparency, lose their sharp outlines, and progress in various degrees towards sphericity. I have drawn attention to this in respect of glycerine in particular, in the *Quarterly Journal of Microscopic Science* for 1873. The transformation of crystals of murexide put up in glycerine is a particularly beautiful phenomenon, exquisite tufts of black-looking needles growing at the expense of the brilliant purple prisms.

"When we seek the causes of the spheroidal modification, several possible influences present themselves for consideration.

"(1) *The 'Nascent' State* . . . a review of the behavior of the various salts and matrices favors the idea that the nascent modification of substances is an important element in the production of changes of form. This I have pointed out in earlier papers in relation to uric acid, which is certainly precipitated in colloid form when freshly dispossessed of its combinations by other acids. The colloid state has been called its hydrate by Prout; and, while in aqueous solutions it is short-lived, in colloidal solutions or mixtures it endures for a long time. An analysis of the phenomena in a great number of experiments made with various media makes me inclined to believe that hydrated colloids and very strong solutions of very soluble salts have the power of prolonging the colloid state of certain crystals. In respect of the present inquiry, any prolongation of the colloidal state in substances precipitated must favor the production of spheroidal forms. Our knowledge, indeed, of the ways of higher colloids must be admitted to be elementary. Of the albumens, for instance, we know really little more than their rough chemical reactions; and though the reports of various societies embalm fragments of what may be more properly called their natural history, most of these fragments remain unnoticed or useless. Whenever these fragments shall be put together and supplemented by further investigation, the connection between colloidality and hydration will be required to be carefully worked out. With this the meaning of the 'nascent' state of substances will form a congenial subject of inquiry; and at present the probability suggests

itself to me that the nascent state is allied to or even identical with the colloid state of matters. Just as chemical substances may be either gaseous, fluid, or solid, as some are commonly seen in all states, some only in one, while many which we are accustomed to see in only one may by special experiments be brought into the other forms, so it seems to me probable that all matters, when deposited from solution, or otherwise assuming a solid form, a liquid or gaseous form, have, or tend to have, a colloid and a crystalloid stage, both of which may be well marked, as in silica, or one of which may be more marked than the other, as in uric acid or only one of which may at present be recognized, as in chloride or zinc on one side, in albumen on the other. . . .

"(2) *Hydration.* Professor Guthrie . . . throws out a suggestion that the partial dehydration of sulfate of copper has to do with the forms found in the evaporated gelatine solution . . . But . . . sulfate of barium contains no water, yet undergoes the spheroidal change in the most complete way. The same may be said of carbonate of barium, of carbonate and sulfate of strontium, and of cholesterin, all of which readily form spheres. . . . Dehydration, partial or complete, can cover, therefore, only part of the facts.

"(3) *Crystalline Form.* I have sought in vain for any indications of any difference in the tendency to sphericity, or in the modifications of spheroidal form assumed by different substances, which might be attributed to their belonging to one or other group of crystalline forms.

"(4) *Relative Solubility.* The best spheres are certainly obtained when substances of little or no solubility are deposited by double decomposition; and when a very moderate degree of insolubility is reached, as in triple phosphate, spheroidal forms are only with great difficulty obtained by that process. But by evaporation (Guthrie), and by deposition from hot, concentrated solutions (nitrate of urea, ferrocyanide of potassium) very soluble matters may be made spheroidal. . . .

"(5) *Influence of the Colloid.* . . . The assumption of the spheroidal state, and the throwing off of the crystalline state, are both consistent with the idea of a state of movement possessing the molecules, engrossing them so fully as to render them insusceptible to attractions by which, being at rest, they would be held and controlled. . . .

"Graham has spoken of colloid as the dynamic form of matter. The constituents of their large molecules are in a perpetual movement or strain tending to ultimate rest in crystallinity, either by isomeric change or by decomposition. The viscosity characteristic of them in their most perfect state, instead of appearing to me as a peculiarity related to their animal and vegetable origin, is partly due to the size and immobility of their chemical molecule, partly also to their intes-

tinal* movements, belonging so far to the same class of conditions as the spheroidal state of water. . . .

"The idea of a 'combination' between the colloid and the crystalloid as constituting an essential part of the whole phenomenon is insisted upon by both Mr. Rainey and Professor Hartung. If by this is meant chemical combination, the idea does not seem to be to be well founded. The observation of Professor Hartung that albumen undergoes a chemical change where the spheres are formed—an observation which agrees with what occurs in urinary calculi—does indeed go to show that a chemical interaction goes on between the two substances. This might be expected to occur when they have been so intimately commingled. But the fact that variations in the density of the colloid solutions produce the phenomena of molecular disintegration alone shows the union to be of physical and not of chemical nature; the history of long-formed spheres, of urinary and of cholesterin calculi in particular, shows that in process of time there is an actual mechanical separation of the colloid from the crystalloid within the substance of the spheres, without loss of the spherical form, so far as it is possessed by the whole mass. . . .† The quantity also of colloid present in a sphere is extremely small in proportion to the crystalloid . . . Such a fact, it is true, is not decisive, but it is at least opposed to the ordinary laws of combination between colloids and crystalloids, a small proportion of the latter usually combining with a large proportion of the former. And, if a combination takes place, why is it limited only to an area of the colloid collimitary with the area of the densely-packed saline matter?"

Dr. Ord then suggests that the relative compactness of bone in different classes of vertebrates depends upon three factors: (a) the nature of the colloid matrix; (b) the nature of the earthy salts; (c) the temperature of the body. Greatest compaction is obtained with albuminous matrix, with a mixture of calcium carbonate and phosphate, and with the highest temperature, as in birds. He adds that bone "may be excavated by a process of molecular disintegration set going by a variation in the constitution of the colloid matrix." (This suggests that enzymic attack on bone collagen might be involved in osteomalacia and pregnancy.)

My own early experiments (1907) speedily showed that the nature of the form developed by any salt depends greatly upon the kind of colloid admixed with its solution, and that with the same colloid different salts give different forms. Thus a solution of one part of sodium chloride, one part of sodium carbonate, and

* This word means "of or belonging to the inner parts." J. A.

† See paper on concretions by Dr. L. Lichtwitz, in Alexander's "Colloid Chemistry," Vol. V, Reinhold Publishing Corp., 1944.

one-tenth part of gum arabic in ten parts of water, when spread on a microscope slide and allowed to dry, showed in some places a "flowering plant," with graceful stems (carbonate) and characteristic four-petalled flowers (chloride). These colloid-crystal effects have been suggested as aids in diagnosis, because subtle changes in body fluids often affect protective action. This principle is the basis of the Lange colloidal gold test for syphilis, which registers differences in the protective action of spinal fluid on highly sensitive gold hydrosols.[18] Dr. Karl Landsteiner (Nobel prize, 1930) begins his book "The Specificity of Serological Reactions" (1936) as follows: "The morphological aspects of plant and animal species form the chief subject of the natural sciences and are the criteria for their classification. But not until recently has it been recognized that in living organisms, as in the realm of crystals, chemical differences parallel the variations in structure."

Colloidal Protection

This important principle has been utilized from remote antiquity by practical people who, unhandicapped by teachings of what is orthodox to do or to observe, frequently make discoveries that surprise scientists and theorists. The late Dr. Edward G. Acheson put it about like this: "It is often the man that does not know any better who does the thing that can't be done. The poor fool does not know that it can't be done—so he goes ahead and does it." The percentage of success of the practical man is commonly below that of the trained scientist; but because of close daily contact with practical problems of life, and also their tremendous numerical superiority, practical men, *in the aggregate*, have in many cases made important advances, which later became of theoretical value. When Lord Kelvin's son drove a golf ball farther than theoretical calculations would allow, his father was led to consider the overlooked factor of the spin of the ball.

From time immemorial the highly effective protective colloid gelatin (or glue) has been used to deflocculate the carbon in Indian or Chinese ink—*because it worked.* We have recently synthesized ephedrine, which we used to secure from *Ma-Huang*, a plant illustrated in the ancient Chinese pharmacopeia.[19] The ancient Egyptians, whose extensive technological knowledge was outlined, e.g., by Sir John Gardner Wilkinson nearly a century ago,[20] used gum (probably acacia) for making their water inks; they also made their clay workable, like that of Babylonia, by infusions of straw, as

indicated in Exodus V. Philippine natives, when panning for gold, often squirt the juice of "gogo bark," which they chew, into the pan to deflocculate and wash away the accompanying clay. About forty years ago, E. G. Acheson obtained patents for "Egyptianizing" clay by using alkaline tannin solutions, etc. The *aurum potabile* of the alchemists was made by reducing solutions of gold (chloride) in the presence of stabilizing ethereal oils, and as early as 1794 silk was dyed with colloidal gold. In his "Lehrbuch" (1844) Berzelius gives recipes for making several shades of colloidal gold; and as early as 1821 isinglass, egg albumen, and starch were used for this purpose.

What Professor Richard Zsigmondy (Nobel prize, 1927) considered to be the first example of protective action, *recognized as such*,[21] was referred to by Thomas Graham.[22] He stated that crude caramel, porduced by heating raw sugar to 210-220° C, when dialyzed, allows a colored substance to pass through, while the material richest in carbon remains behind in the dialyzer. A 10 per cent solution of this residue is gum-like and forms a weak jelly, which is completely soluble in water. Evaporated in a vacuum, it yields a tough, black, elastic, shining mass, which, when thoroughly dry, can be heated to 120° C and still remain completely soluble. If, however, the first solution is evaporated to dryness on a water bath, it becomes insoluble. Both the soluble and the insoluble caramel have the same empirical formula. Liquid caramel is tasteless, neutral in reaction, and extremely sensitive to crystalloid reagents. Traces of mineral acids, alkali salts, and alcohol make it pectous, and the brownish-black, powdery substance yielded by the precipitated caramel is insoluble in both hot and cold water, though it may be rendered soluble again by dilute potash.

Graham then states: "The presence of sugar and of the intermediate brown substances protects the liquid caramel in a remarkable degree from the action of crystalloids and accounts for the preceding properties not being observed in crude caramel." Incidentally, Graham refers to the analogy between caramel and anthracite: "Caramelization appears to be the first step in that direction—the beginning of a colloidal transformation to be consummated in the slow lapse of geological ages." This recalls Dopplerite, a brown, amorphous, elastic or jelly-like substance found in peat-beds, and also the so-called "mother-of-coal" sometimes found in mines.

In 1856 Michael Faraday[23] reported the discovery of "jelly" (evidently gelatin or isinglass) as a protective colloid for colloidal gold; and in 1898, Zsigmondy,[21] then unaware of the preceding

work, rediscovered gold sols and used them to measure the relative protective power of various colloids. The "gold number" represents the number of milligrams of protector which just fails to prevent the coagulative color change, from red to violet, of 10 cc of an extremely pure and highly sensitive colloidal gold solution, upon the addition of 1 cc of a 10 per cent solution of sodium chloride. The use of these sols in the Lange test has been mentioned above. The gold numbers of a few protectors is given herewith:

Substance	*Gold Number*
Gelatin	0.005 to 0.01
Amorphous egg albumen	0.03 to 0.06
Crystallized egg albumen	2.0 to 8.0
Fresh egg—white	0.08 to 0.15
Gum arabic	0.5 to 4.0
Dextrin	6.0 to 20.0
Sodium oleate	0.4 to 1.0
Sodium stearate at 100°C	0.01
Sodium stearate at 60°	10.0
Cane sugar	∞

The generally accepted explanation of the mechanism of prottection is that advanced by H. Bechhold,[24] *i.e.* that the protector is adsorbed at the surface of the protected particle. The nature of the outwardly directed electronic fields is evidently an important factor also, for Bechhold later found that certain minimal quantities of protectors sensitize rather than protect. Thus 0.0003 to 0.0001 part of gelatin per million will flocculate gold sols and oil emulsions. This principle is used in the mining industry (flotation processes), and also to flocculate coal slurries.[25] The work of J. Billiter[26] indicates that these traces of protector may bring the colloid particles to the isoelectric point, where, as Sir William B. Hardy has shown they are especially susceptible to flocculation.

Another curious quirk in the effects of adsorbed surface layers becomes evident in other cases where the newly formed surfaces attract each other strongly and stick together. Alexander[27] considered starches as a mixture of substances: (1) amylopectin, the more coherent, less dispersible gel-like portion (now known to contain branched molecules), and (2) amylose, the more soluble or dispersible portion, now known to contain unbranched molecules.[28] In discussing colloidal protection as a factor in the behavior of starches, it was pointed out that the behavior of a substance or a mixture of substances depends largely on the nature and relative proportions of its aggregating and its protective fractions. The term *cohesive colloid* was suggested for

substances where adsorbed layers cause particles to cohere. In starch grains, the less soluble amylopectin seems to coat over the more soluble amylose; but the resolubility of dried boiled starch paste indicates that there conditions are reversed.

Calcium seems to play an important role in establishing the coherence of particles in aqueous media. Thus lime in soils aids in bringing them into a condition of good tilth, described by Sir. E. J. Russell as that "nice crumbly condition suitable for a seed bed." On the other hand, alkaline soils deflocculate and puddle badly. Calcium humate appears to act as a cohesive colloid, especially when the soil dries out, and thus incidentally prevents the winds from blowing away the valuable top soil. The economy of China is affected by such losses, and "Peiping throat" is caused by breathing the begrimed air. A great dust storm swept over New York on May 12th, 1934; and it is estimated that 300,000,000 tons of soil were lifted from drought-parched Western States by a strong northwest wind, and scattered over half of the United States. It must also be recalled that Professor C. Herbst (Heidelberg), who died in 1946 at the age of 80, had found many years ago that, in the absence of calcium, the cells developing from fertilized sea-urchin eggs fail to cohere, and normal development is frustrated.

The work of Wanda K. Farr and collaborators[29] indicates that cellulose contains ellipsoidal crystallites about 1.5 microns long and 1.1 microns wide, cemented together by a pectinous material.

The passage of the much-needed and beneficial Pure Food and Drugs Act in 1906 was largely due to the efforts of Dr. Harvey W. Wiley, Chief Chemist of the U.S. Department of Agriculture. Flushed with deserved victory, he began soon thereafter to issue what he considered to be "standards" for many foods. He extended these rulings to such confections as ice cream, which he declared should contain nothing but cream, sugar and flavor, the latter including fruits and nuts. Faced with the fact that protective colloids (eggs, gelatin, gum, etc.) had commonly been used in making satisfactory ice cream, and also in candies like gumdrops, Wiley held that French ice-cream, always made with eggs, should be sold under the name "frozen custard." He then instigated a test case against a small ice-cream maker in Washington, which was defended by the National Association of Ice Cream Manufacturers. After hearing the evidence on both sides, the Court directed a verdict of "not guilty" in what I am told was the

first defeat for the Government under this Act. In this case I had volunteered my services as expert because I was then interested in gelatin and its uses, and furthermore because my investigations had shown me not only the justice of the manufacturers' position, but also certain scientific data which had an important bearing on the matter.[30]

At that time I had begun to make experiments with protective colloids, and soon noticed the powerful effect of gelatin on crystallization, *e.g.*, on plaster of Paris.[31] Experiments with cow's milk showed that gelatin and gum arabic stabilize it against coagulation by acid and rennin, and ultramicroscopic examination checked the results. On comparing the composition of cow's milk with that of human milk, and separating the "total protein" figure, commonly used, into casein and lactalbumin, the superiority of human milk in the protective colloid lactalbumin became manifest. The fact that cow's milk can be stabilized by protectors is of importance, because large curds, though suitable for a calf, are not readily handled by an infant; and furthermore, casein entraps the fat globules when coagulating, giving greasy curds which are very hard for an infant to digest.[32]

The literature showed that many types of protective colloids have been used for years in many countries in adapting cow's milk to infant feeding. Professor Abraham Jacobi, later President of the American Medical Association, had long advocated[33] the use of gelatin and gum arabic, and stated that asses' milk is "a refuge to which mothers fly when other milk or mixtures are not tolerated." Cereal gruels, dextrinized starch, seaweed (Irish moss), lichens (Iceland moss), and beer (the dark dextrinous beer of Bavaria) are among the many protectors used, and at present, commercial dextrose, and maltose quite high in dextrin content are popular. With milk mixtures high in fat, like ice cream, colloidal protectors are desirable from the digestive standpoint; and many can tolerate an eggnog better than raw milk, with or without the alcoholic noggin.

In the course of these experiments, an attempt was made to simulate milk by forming a precipitate of calcium phosphate in the presence of colloidal protectors. Protected sodium phosphate was mixed with calcium chloride, and *vice versa*, but a stable sol did not result. In following mentally what must take place in the mammary gland, it was realized that as *all* body fluids contain protectors, *both* of the reacting solutions should contain protectors. Thus was born the concept of

double and *plural colloidal protection.* The doubly protected solutions gave a stable calcium phosphate sol, precipitable with acid and with rennin (1908).[34]

Although U. S. Patent No. 1,259,708 was obtained to cover multiple colloidal protection, it was later found that double protection had been used empirically—in fact, it is referred to in the foregoing quotation from Ord's book. The so-called "grainless" photographic emulsion of G. Lippmann (Nobel prize, 1908) is made by dividing the protective gelatin equally between the silver nitrate and the halide solutions. M. Carey Lea produced some of his silver "photohaloids" in a similar manner, and Lobry de Bruyn[35] also used gelatin in both reacting solutions.

Plural protection is illustrated in the gluten of wheat flour, which contains a protective colloidal system consisting mainly of the following:

Gliadin, which forms an opalescent colloidal solution in water, precipitated by sodium chloride;

Glutenin, which is insoluble in water or saline solutions, but dissolves in weak acid or alkali, and reprecipitates on neutralization;

Globulin and albumin, which are soluble in sodium chloride solutions.

Salt, used from time immemorial in making bread, and the feeble acidity developed by the yeast help to produce desirable bread-making properties in the protective colloids present by means of what may be called a *cumulative protective system* or mixture. Hard water (sulfates) hardens the gluten, while alkaline water disintegrates it and destroys its elasticity. Even distilled water yields a sticky dough.

The multiform effects arising from the interplay of crystallization forces and specific colloids lead one to suspect that the size, shape, and nature of many biological structures are thus dominated.[36] Plant and animal cells and circulating fluids abound in substances which are capable of acting as protectors. As a result, any precipitate forming by reactions between fluids, or even by catalysis at specific surfaces, would tend to be highly colloidal because of double or multiple colloidal protection, and to remain so unless the adsorbed layers function as cohesive colloids, in which case particulate units (*e.g.,* starch grains, cellulose units and fibers) or tissue structures (*e.g.,* wood, fibrous tissue) may emerge. Naturally, the formation of such structures may also involve other factors—enzymes, for example. J. Wolf and A. Fernbach[37] extracted from green cereals an enzyme, *coagulase,* capable of precipitating starch from its solutions.

Mixtures vs. Pure Substances

Apart from the effects of colloidal protection, mixtures of substances often show surprising properties. A small percentage of carbon (probably forming iron carbide) converts iron into steel. Though pure tin melts at 232° C and pure lead at 327° C "half-and-half" solder melts at 220° C. Very pure iron and vanadium have properties quite different from those tabulated for the ordinary "chemically pure" elements.

H. G. Bungenburg de Jong and H. R. Kruyt[38] found that when certain hydrophilic sols are precipitated by a variety of methods, *e.g.*, by salts, by removal of solubilizing salts, by temperature change, and especially by addition of oppositely charged sols, the "precipitate" often forms viscous droplets which aggregate into a fluid mass called a *coacervate,* instead of forming a solid phase. The phenomenon, termed *coacervation,* had been observed by F. W. Tiebackx,[39] who remarked that the gelatin/gum arabic coagula resembled casein. Strongly adsorbed shells of water are supposed to surround the droplets and to prevent their aggregation. In the water-dispersible emulsions of asphalt now much used in road and airfield construction, the protective aqueous films commonly consist of dispersions of soaps or of colloidal clays.[40]

Practical cooks know that by working butter, lard, or hydrogenated oil into baked goods (pie crusts, cookies, etc.) they are made "short" or tender; and the fats are known as "shortening." The fat gets in between the layers of flour dough and so weakens the final product that it breaks off "short," or is flaky, the latter being especially desirable in pie crust. On the other hand, technologists often use a colloidal substance to make a mixture stronger or more cohesive, *e.g.*, starch, glue, or rosin size in paper. B. W. Zweifach reported[41] that cells in the walls of capillaries are bound together by a calcium protein compound, and that diffusion takes place through this intercellular cement rather than through the cells themselves.

Development of Structures in Living Units (Bionts)

Even in a single cell numerous chemical substances are being produced catalytically, and as these are liberated in the nascent state they are likely to be affected by other newly formed neighbor substances, or by other molecules or particulate units afloat in the cytoplasm of the cell. The chemical or physical combina-

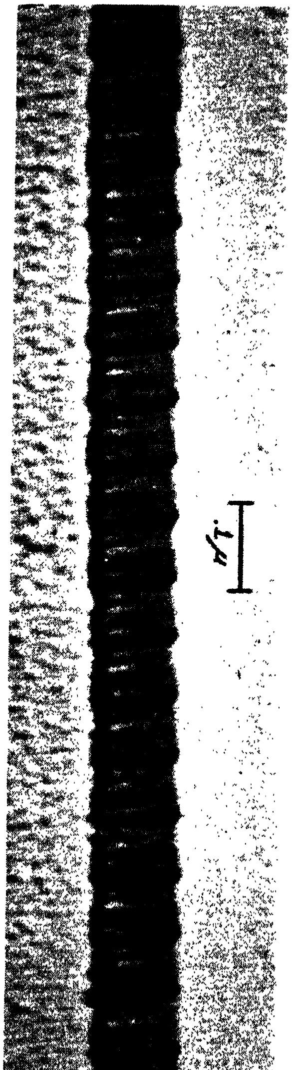

FIGURE 4a. Collagen fibril from human skin corium shadowed with chromium. (× 111,000) [*Courtesy Prof. Francis O. Schmitt, M.I.T.*]

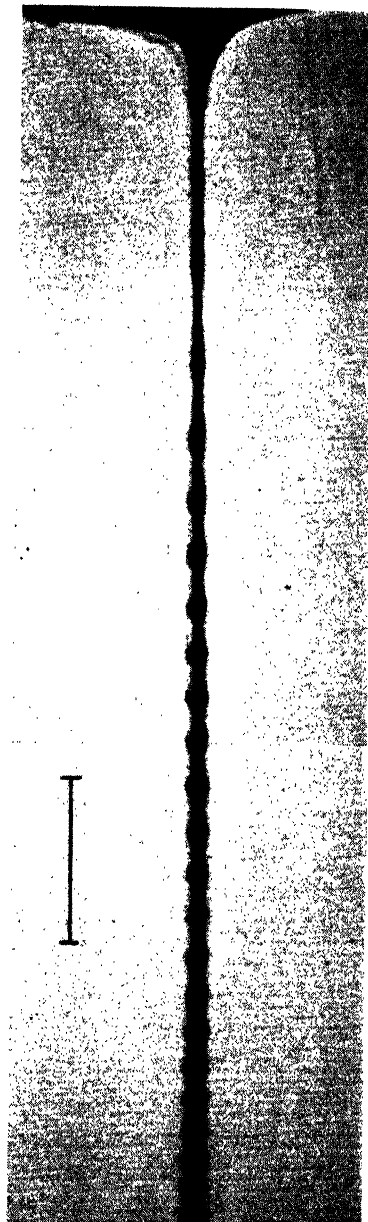

FIGURE 4b. Collagen fibril from rat tail tendon, stretched by peeling back of collodion supporting film. (× 21,000) [Ref: Schmitt, Hall and Jakus, *J. Cell. and Comp. Physiol.*, **20**, 11 (1942)]

tions that result go to make up the various characteristic structures we recognize at higher structural levels, many of which have long been the wonder of microscopists because of the meticulous accuracy of their formation. The electron microscope reveals some of these "vestiges of molecular creation" at submicroscopic levels, as may be seen in Fig. 4, micrographs of collagen. It reveals that this supposedly "homogeneous" substance, in the specimens examined, consists of two substances in regular formation, one of which is more readily stretched than the other. This causes the striations so apparent in the picture. The biocatalysts determine where and what substances shall be formed, and the formation rates; and the subsequent mutual reactions of the substances largely determine what superior structures will emerge.

Some insight as to the further complications that arise at a still higher structural level when numerous cells get together is given by the slime molds. When plenty of food is available, they live singly and divide by fission. After this vegetative stage, the cells gather together in masses, which may number as high as 150,000 cells, and form what is termed a pseudoplasmodium, which moves about as though it were an individual unit. In the case of *Dictyostelium discoideum*[42] the migrating unit stops just before the formation of a spore-bearing stalk (sorocarp). After aggregation of the individual cells (myxameboe) there is no further increase in their number or size; but all further development consists entirely in the integration and subsequent differentiation of the myxameboe already present, which may be of wholly different spore origin and of initially equal potentialities. If the cell masses are broken up in the presence of food (bacteria), they return to the vegetative self-reproducing stage; but if no bacterial food is present, they re-aggregate and develop fruiting structures whose pattern is constant, though the size is in proportion to the cell mass. Different species, thoroughly intermixed in the vegetative stage, aggregate to separate and distinct centers. Though two species of *Dictyostelium* may initially enter the same fruiting organization, they later draw apart and form separate sorocarps, which have a distinctive form for each species. "What is inherited is not a specific type of structure, but the ability of like but discrete and independent units to cooperate in the formation of such a structure."

Just as slowly grown crystals tend to "purify" themselves by elimination of stranger ions or molecules from their space lattices,

so cells of like species tend to segregate, as has so ably been demonstrated by Professor Herbert S. Jennings.[43] When a heterogeneous mixture of bacteria is agglutinated by a heterogeneous mixture of specific antisera, each cluster of bacteria is homogeneous. Apparently each bacterium selectively adsorbs a layer of its own specific antibody, and the bacteria so conditioned cohere or "crystallize" into lattices or clumps because of the specific unions of their new surfaces. The adsorbed antibody appears to act like a cohesive colloid.[44, 45]

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[2] "Colloid Chemistry," Vol. VI, p. 748, New York, Reinhold Pub. Corp., 1946.

[3] Liber XXXV, Par. 185: *Virus alarum sudoresque sedat.*

[4] See, *e.g.*, "Molecular Association," by W. E. S. Turner, in "Colloid Chemistry," Vol. I, pp. 278-287, New York, Reinhold, 1926.

[5] "Properties of Ordinary Water-substance in All Its Phases," A. C. S. Monograph, Reinhold Publishing Corp., 1940.

[6] *J. Chem. Physics*, 1933.

[7] *J. Phys. Chem.*, 1936.

[8] *Phil. Trans. Roy. Soc.*, 1861, **151**, 184.

[9] His original papers were printed in the *Journal of the Russian Chemical Society*, 1906-1908, and a comprehensive paper by him appeared in "Colloid Chemistry, Theoretical and Applied," Vol. I, pp. 27-101, New York, Reinhold, 1926.

[10] See J. Alexander, First Colloid Symposium Monograph, 1923, p. 297, and also paper of H. A. Endres, "The Crystallization of Sulfur in Rubber," in Colloid Chemistry, Vol. I, pp. 808-13, Reinhold Publishing Corp., 1926. Both papers have several illustrations of these phenomena. By ultramicroscopic examination, J. Alexander found that the sulfur-Canada balsam crystals showed ultramicrons at their surface, apparently representing particles unable to find placement in the crystal lattice. Vogelsang expressed the view, also shared by G. Quincke, that globulites are preliminary stages in the formation of crystals. Sir J. S. Flett more recently drew attention to these forms.

[11] *Trans. Faraday Soc.*, 1933, **29**, 990.

[12] For further information in this interesting field, see a paper by G. Friedel on "The Mesomorphic States of Matter," in "Colloid Chemistry," J. Alexander, Vol. I, pp. 102-125 (Reinhold Publishing Corp.), which gives references to other work.

[13] Robert Boyle, "Origin of Forms and Qualities," Oxford, 1666, and Romé de l'Isle, "Crystallographie," I, p. 379, Paris, 1783.

[14] See the papers of Stéphane Leduc (Nantes) and of A. L. Herrera (Mexico) in "Colloid Chemistry," Vol. II, pp. 59-79, and pp. 81-91, Reinhold Publishing Corp., 1928.

[15] *Science*, 1925, **61**, 184.

[16] "On the Influence of Colloids upon Crystalline Form and Cohesion, with Observations on the Structure and Mode of Formation of Urinary and other Calculi," London, 1879.

[17] Ord, Chapter II, "Containing a Discussion of the Causes of Molecular Coales-

cence, and an Application of the Principles Established to Some Structural Phenomena of Living Bodies" (p. 15 *et seq.*).

[18] D. L. and C. T. Morris [*J. Phys. Chem.* (1939), **43**, 623] observed that many biological substances show characteristic crystallization forms with cupric chloride.

[19] A revised edition (the Pên T'sao Kang Mu) was published by Li Shih-chên, 1552-1578.

[20] "Customs and Manners of the Ancient Egyptians," many editions.

[21] See "Colloids and the Ultramicroscope," Eng. trans. by J. Alexander, p. 43, J. Wiley & Son, New York, 1909.

[22] *Phil. Trans. Roy. Soc.*, 1861.

[23] *Phil. Trans.* (1857), p. 145.

[24] *Zeit. phys. Chem.* (1904), **48**, 385.

[25] See "Colloid Chemistry," by J. Alexander, 4th ed., p. 203, New York, D. Van Nostrand Co., 1937.

[26] *Zeit. phys. Chem.* (1905), **51**, 142.

[27] Paper read before a Joint Symposium of the American Chemical Society, New York, April 25th, 1935 (finally published in *Journal of the Society of Chemical Industry*, London, March 13th, 1936.)

[28] See paper on "Recent Advances in Starch Chemistry," by R. M. Hixon and R. E. Rundle, in "Colloid Chemistry," Vol. V., pp. 667-683. Reinhold Publishing Corp., 1944.

[29] See her paper on "Plant Cell Membranes" in "Colloid Chemistry," Vol. V, pp. 610-667, Reinhold Publishing Corp., 1944.

[30] The research director of a large food producer, speaking of the use of the electron microscope in improving the taste, texture and appearance of familiar food products, stated: "Food research scientists have known that the 'feel' of a candy bar as it melts in the mouth has a lot to do with the flavor. A rough grainy texture will suggest a poor flavor. All this is related to the size and shape of the tiny particles which are visible for the first time through the electron microscope." (*N. Y. Times*, Dec. 23rd, 1946.)

For time out of mind practical cooks, as well as ice-cream makers, had been familiar with the use of such colloids as gelatin, gums, eggs, starch, etc., in keeping their products smooth and pleasant-tasting on the tongue, even though they may have been unaware of the scientific principles underlying what their experience had taught them.

[31] See my papers in *Jour. Am. Med. Assn.* (1910), *Journal of the Society of Chemical Industry* (1909), and in *Kolloid Zeitschrift* (1909, 1910).

[32] The subjoined table gives the data for the two kinds of milk, and also for asses' milk.

Kind of Milk	Casein %	Lactalbumin %	Protective Ratio	Fat	Behavior with acid and rennin
Cow	3.02	0.53	0.14	3.64	Readily forms large curds
Woman	1.03	1.26	1.13	3.78	Not readily curded; curds small
Ass	0.67	1.55	2.31	1.64	

[33] "The Intestinal Diseases of Infancy and Early Childhood," New York, 1889.

[34] See "Some Novel Aspects of Colloidal Protection," by J. Alexander, "Colloid Chemistry," Vol. I, pp. 619-627, Reinhold Pub. Corp., 1926.

[35] *Rec. trav. chim. Pays-Bas*, 1900.

[36] J. Alexander, *Science*, (1922), **56**, 323-6.

[37] *Compt. rend.* (1903) **137**, 129.

[38] *Koll. Zeit.* (1929), **50**, 39.

[39] *Koll. Zeit.* (1911), **8**, 198, 238.

[40] See "Colloid Chemistry," Vol. III, pp. 542-6, Reinhold Pub. Corp., 1931.

[41] "Cold Spring Harbor Symposium" (1940), **8**, 216.

[42] Kenneth B. Raper, Third Growth Symposium, 1941, pp. 41-76.

[43] "Colloid Chemistry," Vol. V, pp. 1162-73, Reinhold Pub. Corp., 1944.

[44] The phenomenon of specific clumping has been described by W. W. C. Topley, J. Wilson and J. T. Duncan, *Brit. J. Exptl. Path.* (1935), **16**, 116.

[45] Reference should be made to an extensive paper by Professor Leo Loeb (Washington Univ.) in "Colloid Chemistry," Vol. II (1928), pp. 487-514, in which tissue formation is discussed. He concludes that the basic factors leading to the formation of the most primitive tissues and of agglutination thrombi are the same. Both processes find their prototype in the amoebocyte tissue, and gradually undergo various developments and complications in the more complex classes of organisms. "The primary changes underlying these conditions are localized alterations in the colloidal state of certain constituents of the cells, which are probably protein in nature." The differences in colony form shown by the "rough" and the "smooth" dissociants of pneumonococci appear to be consequent upon the nature of their polysaccharide coats or capsules, as shown by Professor Michael Heidelberger (Columbia University), Dr. O. T. Avery (Rockefeller Inst.) and others.

Chapter 4

The Importance of "Impurities" and Trace Substances

The first question generally asked of a practicing professional chemist is "What will it cost to analyze this sample?" The sample may be anything from a patent medicine to a bit of composition or plastic. The first question the chemist should ask is, "To what use do you expect to put this analysis?" As a rule, the answer will be, "I want to make the same thing." The chemist must then explain that while a routine analysis may be one step toward this objective, it seldom gives the answer; for a final product rarely reveals directly the original raw materials which were used to make it, or the details of the process. Thus, an analysis of a loaf of bread, showing fat, carbohydrate, protein and salt, would hardly tell anyone how to produce a loaf just like it. Part of the original carbohydrate has been fermented away by the yeast, which, in turn, has added somewhat to the protein; and in baking, much of the water used to make the dough and practically all the alcohol formed by the yeast have been driven off. A large Swedish bakery runs most of its delivery automobiles with alcohol condensed from its ovens; and I have heard of a small baker who used to go on an occasional spree with the alcoholic liquid he condensed from oven vapors, on the underside of a large metal sheet cooled by spring water.

Consider an actual case. A client, with an air of great mystery, presented a chemist with a sample of a red paste and asked the cost of an analysis—"that's all." The client admitted that he expected the analysis would show him how to make the product, and on being told that this was most improbable, expressed surprise. At first he refused to say what the material was, where it came from, or for what purpose it was intended; and he was told to find a chemist he could trust. Thereupon, he decided to take the chemist into his confidence, and revealed that he was using the red paste in a special cosmetic, and that it cost him five dollars a pound. This high price made it impossible for him to operate

at a profit, and he hoped to reduce his cost by duplicating the red paste himself. Asked whether it was ideal for the purpose, he admitted it had many deficiencies, but it was the best thing he had found. He was then asked to write out the properties which an ideal color paste should have for his purposes, and was told that instead of trying to duplicate an inferior product, it would be wiser to try to evolve a product as near the ideal as possible. Actually, a superior color material was produced at a raw material cost of nine cents a pound, and this was adapted for use on automatic machinery which could be hired to turn out a completed cosmetic. The cost of the final product, with very small investment, was less than 10 per cent of the original cost.

The actual analytical work in this case contributed but little to its successful conclusion. But in many, if not most cases involving biological questions, very delicate and ingenious analysis lies at the very foundation of the solution of a problem. The presence of small traces of substances may make or mar a product, process, or organism, and exact means must be found for determining them. When students are criticized for not finding, say, one half of one per cent of some element present in an "unknown," they are prone to consider their instructors very exacting and "mean." Even students with a 90 per cent average must be brought to realize that in business or professional life they are expected to be 100 per cent right, every day. Severe penalties await the food manufacturer whose product contains more than the permissible few parts per million of lead, arsenic, copper, etc.; and the lives of patients often depend on proper and correct reports from the laboratory.

The necessity of determining minute percentages has led to ever-increasing refinements in analysis, and physical means are being increasingly called upon to reinforce or to replace gravimetric and volumetric analytical methods.

Nephthelometry, which depends on measuring, often with the "electric eye" or photoelectric cell, the amount of cloudiness produced in a solution by appropriate reagents, can determine, *e.g.*, one part of phosphorus in 333 million parts of water.[1] The quartz spectrograph is used to determine trace impurities which may ruin a metal for certain purposes. Thus Colin J. Smithells states:[2] "The solubility of bismuth in copper is less than 0.002 per cent, and any excess forms brittle films between the copper grains. For most purposes 0.005 per cent of bismuth is the maximum permissible, and where the metal is

to undergo severe cold work this element should be excluded. Antimony exerts a similar influence in copper and its alloys, and brass is practically unworkable if it contains 0.005 per cent of this element." As little as 0.001 per cent of boron in steel necessitates a change in its heat treatment. The crystallization of primary silicon in aluminum/silicon alloys (12 per cent Si) is inhibited by 0.002 to 0.01 per cent of metallic sodium. Such facts indicate the great importance of segregating the different kinds of metal scrap.

The polarograph, electron diffraction, infrared spectrography, the mass spectrometer, and chromatography are all useful in analysis. The last depends upon the differential diffusion of substances down a column of a selected adsorbent, under the influence of a stream of a selected "solvent."

With many biological substances, micro-analysis is resorted to, the total amount of a sample often being less than a milligram, and the substance sought being present only in gammas (1 gamma is 1/1,000,000 milligram). But still more minute traces can be determined by tests with living organisms, *e.g.*, by immunological reactions, or by the effects shown on the growth of yeasts or plant seedlings. Yeast tests show that biotin has a detectable effect in a dilution of one part in 400 *billion*. Dr. Joseph Needham of Cambridge describes[3] an ingenious application of the Cartesian diver[4] ultramicromanometer (originally designed by K. Linderstrøm-Lang) to measure the minute range of gas exchange in the developing gastrula. Needham estimates the approximate sensitivity of the unit as 0.00015 to 0.001 microliter of gas.

The chemical properties of the recently synthesized element plutonium were determined at first on an ultramicrochemical scale. Professor Henry D. Smyth states[5] that "one microgram (1/1,000 mg) is considered sufficient to carry out weighing experiments, titrations, solubility studies, etc. . . . Successful microchemical preparation of some plutonium salts and a study of their properties led to the general conclusion that it was possible to separate plutonium from the other materials in the pile."

The Effects of Trace Substances

In some cases, small amounts of certain substances exert a beneficial effect; the following will serve by way of illustration:

Helpful Traces

Gasoline: 0.06 per cent of tetraethyl lead inhibits "knock."
Rubber: Nitrogenous substances in crude Para facilitate the "cure." Purer plantation rubber demands accelerators.

Condenser Tubes: Traces of arsenic in copper facilitate rolling and greatly reduce corrosion.

Electro-plating: Tiny amounts of "addition agents" in the bath may greatly improve the nature of the deposited metal.

Baking: "Arkady" flour (named after Professor Robert Kennedy Duncan) was mainly calcium sulfate, to aid yeast growth. Sodium bromate, a "yeast food", enables bakers to start with much less yeast, the total savings running into millions of dollars annually.

Lead: Tellurium (about 0.05 per cent) increases corrosion resistance and establishes "work-hardening." Barium (0.08 per cent) makes lead ring like a bell. Calcium (0.03 per cent) increases tensile strength, valuable in sheathing cables.

Brewing: Traces of proteolytic enzymes prevent "cold-cloud" in beer. Water may be "Burtonized" (like that of the River Trent") by traces of lime salts.

Copper: Traces of copper (e.g., from preserving kettles) inhibit the growth of molds in preserves; also the undesirable growth of algae, etc., in reservoirs.

Cast Iron: Tellurium is a powerful carbide stabilizer; 0.0005 per cent is added in making chilled car-wheels.

Technical DDT, the insecticide, contains some substance or substances effective in inhibiting the catalytic release of hydrochloric acid by some insoluble impurity, possibly iron oxide.[6]

On the other hand, here are some cases where traces are harmful:

Troublesome Traces

Foods: Less than one part of copper per million in coffee can be tasted.

Brewing: Minute traces of iron make "ink" with the tannin of the hops, and in high dilution this gives some beers a sickly greenish hue.

Soap: Coconut oil containing one part per million of sulfur gives a soap prone to rancidity.

Lead-burning: Traces of arsenic in an oxy-hydrogen flame prevent good welds. Traces of platinum in lead storage batteries cause self-discharge.

White lead: Traces of silver produce a pinkish discoloration; traces of copper, a greenish tone. Over 0.0015 per cent is objectionable.

Hydrogenation: According to Sabatier, traces of bromine adsorbed from the air of the laboratory, prevent the hydrogenation of phenol; and thiophene in benzene prevents its hydrogenation to cyclohexane.

Dry Batteries: Iron in the pyrolusite ((MnO_2) and copper in the sal ammoniac, both hurt "battery life."

Since we are here most interested in the biological effects of "trace" substances, a few instances of the importance of mineral elements must be mentioned. *Iodine* is an essential constituent of thyroxine (4 atoms per molecule), an oxidation-accelerating hormone of the thyroid gland. *Copper* facilitates the formation of hemoglobin, and is a constituent of many oxidase enzymes (*e.g.*, ascorbic acid oxidase, polyphenol oxidase, tyrosinase, laccase). *Zinc* is essential in carbonic anhydrase, which influences the equilibrium between the H_3CO_3 and CO_2 being carried by the blood. *Cobalt* is a vital element for sheep and cattle (seemingly not for rats). When some New Zealand sheep suffering from "bush sickness" were cured by administration of iron, it was at first thought that the illness was due to iron deficiency; but later investigation proved that a tiny amount of cobalt present in the iron as an impurity was responsible for the cure.

Fluorine in minute amounts seems essential for proper tooth structure, though in humans a slight excess causes trouble, such as black and misshapen teeth. *Vanadium* is an important constituent of the blood pigment of the ascidian *Phallusia mammilata,* and F. Bernheim[7] found that vanadium stimulates oxidation of phospholipids in the liver. *Molybdenum* is being recognized as of biological significance; and some molds will not grow in the absence of traces of *gallium.* Female rats fed on a manganese-deficient diet gave birth to young but lacked maternal instinct; and 97 per cent were unable to suckle their young, which were also neglected by foster mothers, indicating that the latter detected some deficiency. In fowls, manganese is essential to normal development and is the inorganic factor preventing perosis ("slipped tendon"). Professor E. V. McCollum of Johns Hopkins University and his collaborators have done noteworthy work on trace elements in nutrition.[8]

Vitamins and Hormones

These are considered together because they both represent substances essential in minute amounts for the normal development

and functioning of plants and animals, including most microorganisms. Vitamins are as a rule found in foods, while hormones —often called "endocrines"—are produced by the organism, commonly by "glands of internal secretion" whose potent products enter the blood. For many years the criteria of food value were fat, carbohydrate, and protein. Calcium and iron, and later iodine, were regarded as essential minerals. Then it was found that protein foods must contain a certain number of essential amino acids. Since most proteins are deficient in some of these, a variety of proteins in the diet is necessary. Though gelatin is a valuable and readily assimilated protein food, it lacks tryptophane.[9]

In Java, C. Eijkman (Nobel prize, 1929) carried out the pioneer work (1890-97) on polished rice as the cause of beriberi. Professors E. H. Starling and William M. Bayliss of the University of London coined the word "hormone" in 1902; but not until 1911 did Dr. Casimir Funk suggest the word "vitamine" for the *vital amine* found in unpolished rice. The term *vitamin* quickly came to stand for unknown essential trace substances in foods. The isolation, identification, synthesis and biological understanding of vitamins and hormones are brilliant examples of scientific progress, and have led to great advances in nutrition and medicine.[10]

Dr. Wm. J. Robbins, Director, New York Botanical Garden, has kindly prepared the following resume of some of his work.

It is now generally recognized that plants require vitamins and similar growth substances. Most plants synthesize the vitamins they need from simpler and more elementary substances. Only the minority —and these chiefly the lower plants—suffer from vitamin deficiencies; that is, they cannot develop unless the material upon which or in which they grow contains some of the vitamins they require, but are unable to make. Some bacteria, yeasts and filamentous fungi must be supplied with vitamins; others are autotrophic for these substances. All the higher plants, with the possible exception of the saprophytic or parasitic forms, are autotrophic for vitamins, though the isolated roots of some of them have been found to require an extra cellular supply of thiamine, nicotinic acid or pyridoxine for growth.

Schopfer demonstrated in 1934 that *Phycomyces blakesleeanus* did not grow without the presence of thiamine in the culture medium. Robbins and others found that many fungi have one or more vitamin deficiencies. The deficiency may be *complete* (the fungus does not grow in the absence of the vitamin from the medium) or *partial* (the fungus grows slowly in the absence of the vitamin, but more rapidly

if some is present). Both complete and partial deficiencies may be single (for one vitamin) or multiple (for more than one vitamin). The deficiency may be either *absolute* or *conditioned.* An absolute deficiency is one in which no known environmental conditions enable the organism to synthesize the vitamin from simple foods and nutrients; a conditioned deficiency means that under some conditions the organism can synthesize the vitamin and under others it cannot.

The synthetic ability of a fungus for a particular vitamin may be complete, incomplete or zero. For example, *Aspergillus niger* has complete synthetic power for thiamine; it can make this substance if supplied with sugar and mineral salts. On the other hand, *Phytophthora cinnamomi* or *Ceratostomella* from the London plane tree must be supplied with thiamine as such. Between these two extremes of no synthetic power and complete synthetic ability there exist many types of incomplete synthetic power. For example, *Mucor Ramannianus* can make the pyrimidine half of the thiamine molecule but not the thiazole portion; *Ceratostomella pini* can make the thiazole but not the pyramidine part, and *Ceratostomella montium* can combine the two intermediates into thiamine but is incapable of making either.

Among the filamentous fungi deficiencies for thiamine are common, biotin deficiencies are numerous, pyridoxine deficiencies are infrequent, inositol is a growth substance for some and oleic acid for one. Beadle and his associates have developed numerous mutants of *Neurospora* deficient for a variety of vitamins and for specific amino acids.

The potency of many biological trace substances appears from the following: One part of adrenaline in a thousand million can produce a visible effect on the isolated gut of the rabbit. Professor Reid Hunt[11] expressed the results of his experiments with acetylcholine thus: one grain (originally the weight of a single grain of wheat) is capable of lowering the blood pressure of a thousand million cats, but this dosage might not kill a single cat. Professor A. J. Clark[12] observes that the spindle-shaped heart cell of the frog measures about 130 by 10 microns (volume about 3400 cubic microns), and the acetylcholine molecule measures about 1.5 millimicrons. Professor Otto Loewi (Nobel prize 1936) showed that the nerves of the vagus (pneumogastric) reduce heart action by liberating acetylcholine around the heart cells, and Clark estimates that a few thousand of these molecules are sufficient to depress a single cell. "The relation in size is similar to that between a large whale (100 tons) and a midge (⅓ mg)."

The tables of vitamins and hormones presented at the end of

this chapter give the various vitamins and hormones at present known, with some information as to their nature and action, but with no suggestion of finality in this continually advancing field. Professor David E. Green of Columbia University, in a recent address[18] on physiological function from the standpoint of enzyme chemistry, stated:

"Vitamins B_1, B_2, B_6, and the P-P [pellagra-preventive] factor, have all been shown to be the prosthetic groups of certain enzyme systems. When these vitamins are not available in the diet, the active enzymes cannot be formed in the cell. In consequence of the failure of these enzyme systems to function properly, an abnormal physiological situation develops, which, if uncorrected, will lead to death. Which organ first registers the effect of a particular deficiency is determined by the amount of the reserves of enzymes containing the vitamin and by the relative importance of this set of enzymes in the economy of the organ. Thus, in B_1 deficiency in the pigeon, the brain is the first organ to register disturbed function, presumably because there are no reserves of this vitamin in the brain and because the enzyme formed by the vitamin plays a key role in the metabolism of the brain. One may well raise the point that if, as in avitaminosis, the causal link between the physiological disturbance and the effect on enzyme systems is unquestioned, then surely there is a good case for assuming the same link between normal physiology and enzyme systems."

"The study of endocrines has always been one of the most active fields of physiological investigation, and it is of interest to inquire to what extent hormones can be related to enzyme phenomena. Until quite recently, hormones were held up as notable exceptions to the rule that substances which act at high dilutions must be enzymes or parts of enzymes, or must specifically affect some enzyme system. Recent research, however, fails to confirm the hormones as exceptions to the enzyme-trace substance thesis—the epoch-making discovery of Cori and his group[14] that one of the hormones of the anterior pitituary inhibits the action of hexokinase, and that this inhibition, in turn, is released by insulin. We have here a clear blueprint for the way in which hormone antagonism can be effected. A key enzyme system which controls some metabolic process can be regulated by a set of hormones, one of which inhibits, while the other releases the inhibition. All students of endocrinology have long been aware that hormones regulate metabolic processes, and it is not surprising to find in one instance, at any rate, that the regulation operates at the

level of enzyme systems. Houssay and his colleagues in the Argentine have presented cogent evidence that renin, a kidney hormone, is a type of proteolytic enzyme which hydrolyzes one of the plasma proteins to form a pressor substance. In this substance, the hormone regulates metabolic processes by actually assuming an enzymatic role."

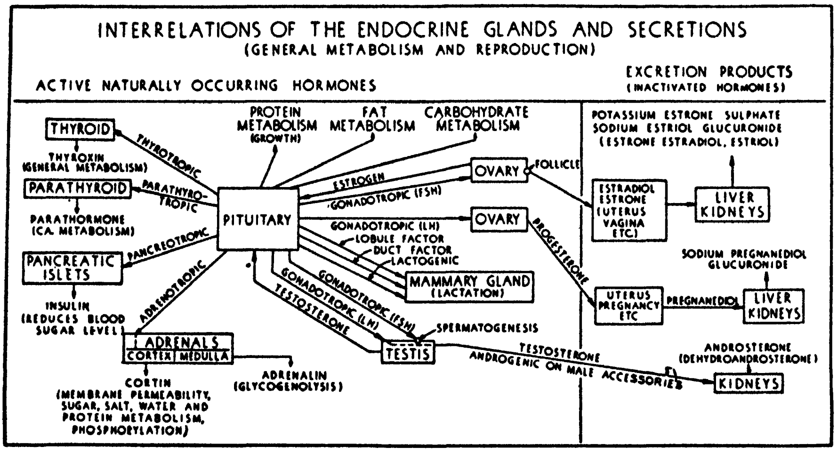

FIGURE 5. The position of the pituitary in relation to the hormonal configuration. (*Courtesy Dennis T. Mayer, Missouri Agr. Exp. Sta.* From "Bioenergetics and Growth" by Brody, Reinhold Publishing Corp., N. Y.)

The profound and diverse influences of the pituitary hormones indicated in Figure 5 led Sir Walter Langdon-Brown[15] to call this gland "the leader of the endocrine orchestra." But in a recent address,[16] he is reported as saying that it later transpired that the hypothalamus holds the still more important rank of "conductor of the endocrine orchestra." The mechanism of hormone action is discussed at length by Oliver Kamm and D. K. Kitchen.[17]

Vitamin research continues to reveal new trace substances important to the welfare of living things, and it may be that some of these are a bit more complicated than at first thought. C. D. Robeson and J. G. Baxter[18] report a naturally occurring isomer of vitamin A (termed by them Neovitamin A) isolated from fish-liver oil where it constitutes about one-third of the total "vitamin A." In rats both isomers have the same biological potency, for rats can transform Neovitamin A into vitamin A. Catalytic interconversion of the anthraquinone carboxylate esters of the two vitamins was accomplished *in vitro* by a trace of iodine in benzene solution. Since iodine catalysts (among many others) are

active in many living things, we must envisage the possibility that vitamins and hormones may, directly or indirectly, affect one another.

An interesting example of hormonal interrelationships appears from recent research on the thyroid gland and the iodine catalysts of the body, which are continually being assembled and released, and when broken down are salvaged and reconstituted for re-use, as follows:

(1) Within the gland a catalyst mechanism sets free or secretes a mass of large, non-diffusible particles of thyroglobulin, forming visible masses known as "colloid."

(2) The colloid specifically adsorbs and stores iodine in the form of iodide, diiodotyrosine, and thyroxine, and gradually releases these catalysts into the circulation for service in such cells as may take them up.

(3) The thyroid-stimulating hormone of the anterior pituitary (TSP) stimulates colloid formation, conversion of diiodotyrosine into thyroxin, and release of the latter into the circulation. TSP is of protein nature.

(4) Iodine-containing material restored to the circulation from the cells is recaptured by the colloid and returned to the iodine cycle, apart from small amounts excreted in bile, urine, etc. The actual amount of iodine in circulation at any time is normally very small.

(5) Thiouracil and similar goiter-forming substances inhibit absorption of iodine and its conversion into diiodotyrosine and thyroxin. The latter process appears to occur in two steps: (a) oxidative liberation of iodine to combine with tyrosyl radicles; (b) coupling of two diiodotyrosyl groups to form thyroxin residues.

In addition to serving either directly as catalysts, or as carriers or prosthetic groups in enzymes, trace substances may affect the permeability of membranes (septa), through which the raw materials and reaction products of the catalysts must diffuse, and thus influence reaction velocities. Working through the endocrine, nervous, or circulatory systems, such stimuli, though physically small, may trigger off vitally important reactions.

HORMONES OR ENDOCRINES

Glandular Source	Name, Nature	Chief Physiological Action	Common Consequences of Excess (+) or Lack (−)
Thyroid	Thyroxine	Speeds metabolism, especially oxidation	+Exophthalmic goiter (Graves' disease) −Myxodema—infantile cretinism
Parathyroids		Governs Ca metabolism	+Hypercalcemia −Tetany
Adrenals			
Medulla	Epinephrine (Adrenaline)	Vasoconstrictor; raises blood pressure	+High blood pressure, excitement −Low blood pressure, weakness
Cortex	Cortisterone (Cortin)	Electrolyte regulation Stimulates resistance to bacterial infection (C. H. N. Long)	−Addison's disease; progressive anemia, brown skin
Pancreas	Insulin	Controls oxidation and storage of glucose	+Hypoglycemia −Diabetes mellitis
Intestinal Tract			
Stomach	Gastrin	Stimulates gastric and pancreatic secretion	
Duodenum	Secretin Choleocystokinin	Stimulates flow of bile	Bile makes gut more permeable to chyle
Gonads			
Ovary	Estrone (theelin, folliculin) Estradiol, theelol (in tissue)	Estrus	
Corpus luteum	Progesterone, Luteosterone	Prepares uterus Gestation hormone	
Testes	Testosterone Androsterone	Develop secondary male characteristics	
Nerves			
Parasympathetic	Acetylcholine	Quiets smooth muscle	−Tremor
Sympathetic	Sympathin	Excites smooth muscle	

HORMONES OR ENDOCRINES (Continued)

Glandular Source	Name, Nature	Chief Physiological Action	Common Consequences of Excess (+) or Lack (−)
Pituitary			
Anterior	(1) Somatotropic	Stimulates cellular growth	
	(2) Gonadotropic	Stimulates and maintains corpus-luteum	This group has effects on carbohydrate metabolism (Houssay)
	(3) Thyrotropic	Stimulates thyroid gland	
	(4) Corticotropic (Adrenotropic)	Balances effects of (1) (H. M. Evans)	
	(5) Luteotropic	Stimulates lactation	
Posterior	(1) Pressor hormone	Antidiuretic	—Diabetes insipidus
	(2) Oxytocic hormone		
Kidney	Renin	Hydrolyzes plasma proteins	
Thymus			
Pineal Gland			

Hormomers are substances which exercise physiological effects similar to those of the main naturally occurring hormones, even though they may differ greatly in chemical structure. Thus, diethylstilbestrol produces some results similar to those of progesterone. For further details, see "The Mechanism of Hormone Action" by Oliver Kamm and D. K. Kitchen in Alexander's "Colloid Chemistry, Theoretical and Applied," Vol. V, pp. 749-752 (Reinhold Pub. Corp., 1944); also "Bioenergetics and Growth," by S. Brody (Reinhold Pub. Corp., 1945).

VITAMINS

Kind	Chemical Nature	Deficiency May Cause	Good Food Sources
Fat-Soluble Group			
A—anti-ophthalmic	½ molecule of beta-carotene (P. Karrer)	Xerophthalmia Night-blindness Stunted Growth	Fish oils, liver, cream, fresh vegetables
D—antirachitic	7-dehydrocholestrol Ergosterol is precursor	Rickets (in young) Osteomalacia (in adults)	Fish oils, milk eggs Sunlight to activate precursors
E—Anti-sterility	Alpha-tocopherol	Sterility (in rats) Gonad degeneration (male) No placenta forms (female)	Wheat germ, milk, eggs, meat, lettuce, water-cress
K—anti-hemorrhagic	Naphthoquinones (several)	Prothrombin not formed, blood-clotting fails	
F—fatty acids	Linolenic acid Linoleic acid Arachidonic	Various skin and kidney abnormalities (in rats)	Corn oil, linseed oil
C—anti-scorbutic	Ascorbic acid	Scurvy, fatigue, soft gums	Citrus fruits, peppers, spinach, water-cress
B-vitamin Complex ("*Bios*")			
(1) Thiamine (B_1) (R. R. Williams)		Beri-beri, polyneuritis, loss of appetite	Wheat, eggs, milk, yeast, fruit, vegetables
(2) Riboflavine (B_2) (A. Szent Györgyi)	6.7-dimethyl-9-*d*-ribytyl isoalloxazine	Cheilosis (sore lips)	
(3) Nicotinic Acid (C. A. Elvehjem)	3-pyridine-3-carboxylic acid (its amide functions)	Pellagra ("rough skin")	
(4) Pyridoxine (B_6)	1-methyl-2-hydroxy-3·4-hydroxymethyl hydrochloride	Dermatitis (in rats, and chicks)	
(5) Pantothenic Acid (R. J. Williams)	$CH_2 \cdot C(CH_3)_2 \cdot CHOH \cdot CO$ (lactone form) combines with β-alanine		
(6) Biotin (H) (H. Kögl, V. du Vigneaud)		Anti-perosis (chicks) Anti-avidin; in rats, lack causes dermatitis	Egg-yolk, milk, liver, yeast
(7) Inositol	Hexahydroxycyclohexane	Dermatitis Alopecia	

VITAMINS (Continued)

Kind	Chemical Nature	Deficiency May Cause	Good Food Sources
(8) *p*-aminobenzoic acid. (S. Ansbacher)		Affects tyrosinase activity (gray hair)	
(9) Choline............	Trimethyl-hydroxyethyl ammonium hydroxide	Constituent of lecithin	
(10) Folic Acid (L. *folium*, leaf)............	Pteroylglutamic acid..................	Bone marrow, bowel troubles, etc.	Liver, yeast
P—Citrin.............. (A. Szent Györgyi)	Permeability factor		
S. Streptogenin......... (D. W. Woolley)	Seems to increase growth rate of microorganisms and animals. Apparently a peptide in proteins (casein); obtained by enzymic digestion		

Vitamers are substances having physiological effects similar to naturally occurring vitamins (Oliver Kamm and D. K. Kitchen, in Alexander's "Colloid Chemistry Theoretical and Applied," Vol. V, p. 741 (Reinhold Pub. Corp., 1944).

Many details regarding folic acid are given in *Ann. N. Y. Acad. Sci.*, 1946, 48, 255-350 (thirteen papers). An "anti-stiffness factor" for guinea pigs is found in raw cream, and especially in cane molasses and cane juice; it apparently regulates calcium phosphate deposition and phosphorus metabolism.

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[1] Kober and Eggerer, *J. Am. Chem. Soc.* (1915), **37**, 2375.

[2] "Impurities in Metals," J. Wiley & Sons, New York, 1928, p. 94.

[3] "Biochemistry and Morphogenesis," pp. 194-6.

[4] Needham points out in a footnote that the Cartesian diver, often sold as a toy under the name of "Cartesian devil," was first described in 1648 in a booklet on the compressibility of water by Raffaele Magiotti, a pupil of Galileo; the book has a diagram of the "diver," which, until this application, had remained a philosophical toy. Descartes did not invent it, but the term "Cartesian" was sometimes applied to ingenious scientific or mechanical apparatus.

[5] Atomic Energy for Military Purposes, 1945, p. 101-2.

[6] Fleck and Haller, *J. Am. Chem. Soc.* (1946), **68**, 142; A. L. Flenner, *ibid.*, **68**, 2399.

[7] *J. Biol. Chem.* (1939), **127**, 353.

[8] See A. T. Shohl, "Mineral Metabolism," Chapter 11, Reinhold Pub. Corp., 1939.

[9] Professor William C. Rose (Univ. of Illinois) and collaborators [*Science* (1937), **86**, 298, and "Nutrition Foundation Symposium," Nov. 13th, 1946] give the following data:

Final Classification of the Amino Acids with Respect to their Growth Effects in the Rat

Essential	Non-Essential
Lysine	Glycine
Tryptophane	Alanine
Histidine	Serine
Phenylalanine	Aspartic acid
Leucine	Glutamic acid
Isoleucine	Proline
Threonine	Hydroxyproline
Methionine	Tyrosine
Valine	Cystine
Arginine*	Citrulline

* Arginine can be synthesized by the organism of the rat, but not at a *sufficiently* rapid rate to meet the demands of *maximum growth.*

[10] The following Nobel prizes were awarded for work in these fields: A. Windaus (1928), A. Harden (1929), O. Warburg (1931), Paul Karrer (1937), Albert von Szent-Györgyi (1937), Richard Kuhn (1938), A. Butenandt (1939), L. Ruzicka (1939), Edward A. Doisy (1943), Hendrik Dam (1943), Arturi Wirtanen (1945).

[11] *Science* (1930), **72**, 528.

[12] *Chemistry and Industry* (1930), **49**, 533.

[13] *Ann. N. Y. Acad. of Sci.*, Dec. 15th (1946) **47**, 515-9.

[14] W. H. Price, Carl F. Cori and Sidney P. Colowick, *J. Biol. Chem.*, (1945), **160**, 633.

[15] *Practitioner* (1931), **127**, 614.

[16] Abstract in *J. Am. Med. Assn.* (1946), **131**, 1238.

[17] *Reference given in accompanying tables.*

[18] *J. Am. Chem. Soc.* (1947), **69**, 136-141.

Chapter 5

What Are Living Units?

Many authorities maintain that it is not possible to draw a line of cleavage between living and non-living entities. The task is complicated by the fact that nature is so rich in examples and so gradual in most changes, that it is hard to fit all the data into the rigid frame of any definition. Nevertheless, we must first try to define what we mean by "living" units or entities.

In its broadest sense a living unit or entity is one that can direct chemical changes by catalysis,* and at the same time reproduce itself by autocatalysis, that is, by directing the formation of units like itself from other, and usually simpler chemical substances. Among the simplest known living units are genes, and some of the bacteriophages and ultrafiltrable viruses, which in size approximate molecular dimensions. The electron microscope indicates that some bacteriophages ("the bacteria of bacteria") and some viruses (*e.g.*, that of psittacosis, or "parrot fever") are tiny organisms.

Figure 7, prepared by Dr. W. M. Stanley (Nobel Prize, 1946) shows that some viruses and bacteriophages approach molecular dimensions. *See also Figure 8,* an electron micrograph of tobacco mosaic virus.

Organisms are known which can synthesize their own organic compounds from inorganic substances. These are called *autotrophs.* The best known of this group are chlorophyll-containing plants, including algae, which by photosynthesis form organic compounds from carbon dioxide. The *autotrophic bacteria* may live in the absence of light, are generally microscopic, and are not distinguishable morphologically from other bacteria; but they derive their energy from the oxidation of inorganic substances and utilize it to reduce carbon dioxide to organic compounds. Intermediate between the autotrophic bacteria

* Catalysis is discussed in the next chapter. In brief, it is the process whereby a specific particulate unit or surface (the catalyst) continuously brings about chemical union, breakdown, or structural change in other units as a result of very close contact or approach, under suitable conditions.

FIGURE 6. Electron micrograph of E. coli in the presence of bacteriophage particles. (*Courtesy RCA Laboratories, Princeton, N. J.*)

and chlorophyll-containing plants are the purple bacteria, which require both hydrogen sulfide and light for their development.[1] Robert L. Starkey classes known autotrophic bacteria as follows:

A. Bacteria which oxidize compounds of nitrogen
 (a) ammonia to nitrate (*Nitrosomonas, Nitrosococcus*)
 (b) nitrite to nitrate (*Nitrobacter*)
B. Bacteria which oxidize sulfur and compounds of sulfur
 (a) Simple bacteria (genus *Thiobacillus*)
  (1) Strictly autotrophic
   a) Aerobic

	Diameter or width X length in mμ
Red blood cells	7500
B. prodigiosus (Serratia marcescens)	750
Rickettsia	475
Psittacosis	450
Canary pox	260 x 310
Myxoma	230 x 290
Vaccinia	210 x 260
Pleuro-pneumonia organism	150
Pseudo rabies	150
Herpes simplex	150
Rabies fixe	125
Influenza	115
Newcastle disease	115
Vesicular stomatitis	100
Staphylococcus bacteriophage	100
Fowl Plague	90
T_2 coli bacteriophage	60 x 80
Chicken tumor I	70
Equine encephalomyelitis	50
T_3 coli bacteriophage	45
Rabbit papilloma (Shope)	44
Pneumonia virus of mice	40
Tobacco mosaic and strains	15 x 280
Latent mosaic of potato	10 x 525
Gene (Muller's est. of max. size)	20 x 125
Southern bean mosaic	31
Rift valley fever	30
Tomato bushy stunt	26
Poliomyelitis (Lansing)	25
Hemocyanin molecule (Busycon)	22
Yellow fever	22
Hemocyanin molecule (Octopus)	20
Louping ill	19
Tobacco ring spot	19
Japanese B encephalitis	18
Alfalfa mosaic	17
Tobacco necrosis	16
Foot-and-mouth disease	10
Silkworm jaundice	10
Hemoglobin molecule (Horse)	3 x 15
Egg albumin molecule	2.5 x 10

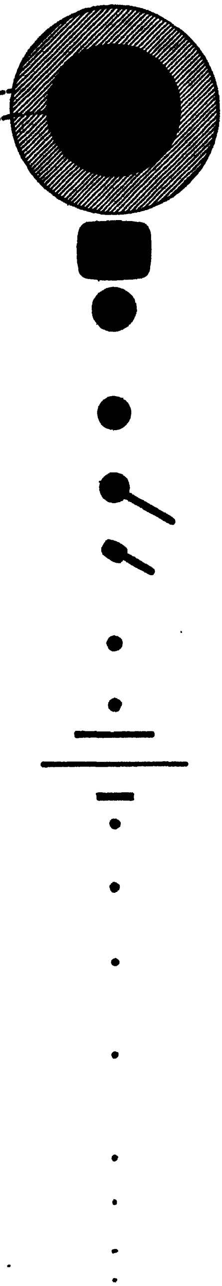

FIGURE 7. Approximate sizes of viruses and reference materials. (Courtesy W. M. Stanley, *Chemical and Engineering News,* 1947)

1) Develop at reactions close to neutrality (species *Th. thioparus*, Beijerinck)
2) Develop under very acid conditions (species *Th. thiooxidans*, Waksman and Joffe)

b) Anaerobic (species *Th. denitrificans*, Beijerinck)

(2) Facultative autotrophic

a) Facultative anaerobic (species of Trautwein)

(b) Higher bacteria (complex in morphology)

(1) Colorless (includes genera *Beggiatoa, Thiothrix, Thioploca, Achromatium, Thiophysa, Thiovulum,* and *Thiospira)*

(2) Pigmented red or purple bacteria (over 16 genera)

C. Bacteria which oxidize ferrous or manganeous compounds

(a) Simple bacteria

(1) Long excretion filaments (genus *Gallionella*)

(2) Coccoid or oval shapes in masses (*Siderocapsa, Sideromonas*)

(b) Filamentous bacteria (genera *Leptothrix, Crenothrix*)

D. Bacteria which oxidize hydrogen.

S. Winogradsky[2] pioneered in this field; first studying certain higher sulfur bacteria, later iron bacteria and nitrifying organisms. The latter convert urine, etc. into nitrates from which was made saltpeter, a vital ingredient of gunpowder.

All known living units appear to have the further power of undergoing generally infrequent but marked deviations from their usual composition, structure and function; and in some cases these changes alter or modify their ability to direct catalytically the formation and decomposition of various chemical substances, and are, furthermore, passed on hereditarily when the living unit reproduces itself by autocatalysis.

Many geneticists have held, and a few may still believe, that *all* heritable changes are due to abrupt changes in genes ("mutations"). This notion was apparently supported by the pioneer work of Professor Herman J. Muller (Nobel prize, 1946) and his followers on the effects produced by subjecting to controlled x-ray treatment eggs, sperms, and seeds. Later it appeared that they might be caused by chromosomal rearrangements rather than changes in gene structure. These matters are considered further in Chapter 8.

Increasing experimental evidence is being found for the view that—apart from heritable changes in the genes and chromosomes, admittedly the main carriers of heredity—the cytoplasm, that pool of "protoplasm" with its numerous enzymic catalysts, mito-

chondria, chloroplasts, and other particulate inclusions, may also undergo changes which are sometimes heritably transmitted. In nature, mutations generally appear in a random, haphazard manner, giving what nurserymen term "sports." The orderly and regular development of individual organisms from seeds or fertilized eggs indicates a more positive and dependable mechanism for the differentiation of the original germ cell (zygote) into various types of cells, tissues and organs. In fact, differentiated cells (heart, leucocytes, or cancer) in tissue culture may continue to reproduce themselves true to their differentiated type, though dedifferentiation or reversion to some other type (*e.g.*, "embryonic" type) may occur.

The essential characteristic of true life units is their ability to increase the number of their kind by direction of chemical changes, whereby non-living and generally simpler atoms, molecules and masses are transformed into the catalytic and reproductive structure of the living entities. Incidentally, besides the catalyzed molecules incorporated into the living unit or into its autocatalyzed "descendants," many other molecules may be discharged into the surrounding medium or *milieu,** and this molecular waste is useful and often essential to other living things. Thus many thousands of tons of urea are being excreted daily into the world's chemical economy, besides large amounts of many other substances. *Non-living entities,* such as molecules of benzene or sulfuric acid, do not increase in number through the direct catalytic action of pre-existing or "parent" molecules of benzene or sulfuric acid, which are typical non-living entities.

Functional Life: Living vs. Dead

In a more restricted sense of the term, we may designate as "living" any cell, tissue, or organ from a living unit while it is carrying on the processes it ordinarily did during the functional life of that unit. Thus a frog's heart may be kept "alive" and beating after removal, though the frog is functionally dead. The death of the organism as a whole (*somatic death*) is more slowly followed by *cellular death,* as the blood ceases to circulate and the cells perish in the products of their own metabolism. By quick and careful work, living tissues from dead persons have been successfully transplanted onto the living. Utilizing and improv-

* We have no English equivalent for this French term, and it has been adopted into English by scientists.

ing upon tissue culture methods originated in the laboratory of Professor Ross G. Harrison of Yale University. Dr. Alexis Carrel (Nobel prize, 1912) and his collaborators at the Rockefeller Institute for Medical Research were able to keep alive a culture of chicken heart cells in an embryonic juice medium for over a third of a century. Professor Robert Chambers of New York University made moving-picture films of a group of heart-muscle cells beating rhythmically in tissue culture, while from the other side of the culture chamber rapidly growing cancer cells invaded

FIGURE 8. Electron micrograph of tobacco mosaic virus (× 18,000). (*Courtesy University of Toronto*)

the "heart" and interfered with its contraction as a unit (fibrillation).

Where large numbers of cells are associated in community life, as in multicellular plants and animals, most of the cells become so highly specialized or differentiated (as in tissues, organs, or blood), that they are quite incapable of carrying on an independent existence. Therefore, if one organ or even a small group of cells fails, the whole organism may perish. The deadly prussic or hydrocyanic acid produces speedy death by inactivating certain cells in the medulla which control respiration and heartbeat. It is not generally recognized that sulfuretted hydrogen has an action similar to that of prussic acid; but in some respects it is even more dangerous because its fixation is irreversible. The

use of cylinders of pure sulfuretted hydrogen to reinforce the feeble content of some "sulfur springs" may, in careless hands, cause deaths involving serious legal responsibility.

Some important bodily failures or injuries may not be fatal, providing the deficiencies are made good artificially. Thus diabetics who would otherwise die may be kept alive by regular injections of insulin. In a case recently reported, a pregnant woman, who had for six months remained unconscious and practically helpless following a head injury, gave birth to a normal healthy child.

In the more restricted sense of "living" mentioned above, sterile, castrated or isolated males or females are said to be alive, though incapable of reproduction. About ten years ago the heath-hen vanished, like the dodo and the great auk, with the death of the sole surviving specimen at Martha's Vineyard. The passenger pigeon is also extinct, though early Americans saw flocks containing enormous numbers. On the other hand, given the proper chemical and physical environment, *i.e.*, proper food, milieu, and where necessary, proper mates, some living units may produce with great rapidity. It has been estimated that the ocean would soon be a solid mass of herring, if all the eggs produced developed into mature fish. It is only disease and the constant competition of various forms of life with one another, whereby unrestrained reproduction is counterbalanced, that permit the great diversity and evolutionary development of elementary living units.

These aspects of the interrelations of living things are considered by the comparatively recently science of *ecology*, which originated in the study of plants, but has been extended to include animals. The devious nature of these interrelations has been illustrated by the correlation mentioned by Darwin between old maids and red clover. Because of the nature of its flower, red clover is pollinated mainly by bumble bees, which build their nests in the ground. Marauding mice prey upon the nests; but cats, which prey upon the mice, are commonly kept by old maids, who thus unwittingly help the red clover. The curiously shaped flower of the "Dutchman's pipe" (*Aristolochia macrophylla*) has its pistil well above the stamens in a long corolla, whose narrow mouth contains many stiff hairs pointing inward. A tiny insect can enter, but is held prisoner until it has effected fertilization of the flower; whereupon the hairs droop and the imprisoned insect

can escape. I have often cut open these flowers and found a tiny fly entrapped within them.

When fig trees were planted in California, they grew splendidly; but the fruit did not mature until a small wasp, *Plastophaga psenes* (Linn.), which fertilizes the flowers, was imported (1890) from Smyrna, cultivated, and released to do this necessary work.

Functional Life: Active vs. Latent

In still another sense we may say that a unit is "alive" if, having ceased to carry on active life processes (catalysis and autocatalysis), it is able to resume them on the reestablishment of suitable conditions. Many plants and animals, *e.g.*, many bacterial spores and protozoa, revive after prolonged desiccation leading to a more or less complete stoppage of active life. The African lung fish has been known to survive as long as four years in a cake of dried mud. Admiral Byrd found in the Antarctic regions primitive plants whose activities began again as the long winter began to yield to the equally long summer; for though high temperatures are fatal to all living things, many organisms revive even after long immersion in liquid air. R. B. Harvey[3] reported finding an alga (*Phormidium*) growing in Beryl Spring (Yellowstone Park) at a temperature of 89° C.

The common notion that all functionally living things must show visible signs of life, such as breathing or motion, is based on experience with humans and many animals. In the case of hibernating animals like bears, hedgehogs, and woodchucks, visible breathing ceases for long periods. Although the irritability of the heart is increased, it beats only enough to maintain a sluggish circulation, and the body temperature approaches that of the surroundings. The animal lives at a much reduced rate on its accumulated reserves of fat and protein, and its slight demand for oxygen seems to be met in part by diffusion. Fakirs and magicians endure temporary burial by a rigid suppression of many bodily processes. Sudden panic, leading to violent muscular activity to escape, would no doubt be quickly fatal. The condition assumed by successful practitioners of this feat seems in some respects to resemble an artificial hiberation.

The vitality of "germs" (microorganisms, spores, etc.) and even of seeds is astonishing. Using rigorous precautions against contamination, Professor Charles B. Lipman, Dean of Graduate Studies, University of California, reported the presence of viable

bacteria in the interior of adobe bricks from old Spanish Missions and from Aztec and Inca ruins, as well as in coal samples taken 1,800 feet below the surface of the earth. He believed that the peat-like coal-forming material of past geologic ages was extremely rich in bacteria, and that some of their spores still survive. He also isolated an autotrophic bacterium from petroleum coming from a well 8,700 feet deep.[4] Confirmation of this work will be welcomed.

To test the viability of seeds, Dr. W. J. Beale of Michigan Agricultural College in 1879 buried 20 different kinds of plant seeds, and had samples taken every five years for test. After forty years' burial, half of the species produced sprouts.[5] In 1902 Dr. J. W. T. Duvel of the U. S. Department of Agriculture buried seeds of 107 species, and found that 69 species grew after 10 years' burial, and 20 species after 20 years' burial. Most hardy were the seeds of wild plants and weeds. Cereal, legume, and cultivated plants generally failed to grow; apparently they depend upon human help to perpetuate themselves.[6]

Becquerel[7] tested 550 species of seeds which had been stored at the Jardin des Plantes in Paris for from 25 to 135 years. Nothing older than 80 years germinated. I have been assured by the late Dr. A. Lucas, chemist of the Cairo Museum, that the so-called viable "mummy wheat," sometimes "found" by native guides in Egyptian tombs, represents recent crop material placed there presumably by the finders. The fraud has been often exposed. R. Whymper and A. Bradley report[8] that a sample of English wheat stored under conditions of desiccation favorable to longevity, showed 69 per cent germination after 32 years' storage. Though exhaustion of the sample (1945) ended this series of tests, the yearly record indicates that an estimated extreme limit of life of 50 years is rather an understatement.

On visiting Palestine a few weeks after unusually heavy rains, I saw a gorgeous, multicolored floral display on the normally bare hills. And in 1930, after one of the rare rainfalls in the desert of Death Valley, California, there was a luxuriant growth of plants, arising from long-dormant seeds, many probably carried there by the winds.

In 1943 a slightly spotted greenish cast was noticed on the grayish limestone wall of the corridor opposite the elevator shaft in the Carlsbad Caverns, New Mexico, about 750 feet below the surface; it gradually spread and became grass-green. In 1941 there

had been an unprecedented rainfall in May; and in September 21.25 inches of rain fell in nearby Dark Canyon, the local Ranger Station reporting 17 inches as falling within a few hours. Apparently enough water seeped through imperfections in some 500 to 800 feet of rock to moisten the exposed rock wall and perhaps carry some spores of algae; or the spores may have been carried in by visitors or by down-draft air currents. An electric lamp, burning seven hours daily, supplied the needed light. Several other growths of algae were also found.[9]

Particular mention must be made of the extremely resistant spores of the anthrax bacillus, which produces a highly contagious and usually fatal disease in men and animals. Whole fields have become a source of infection because of the burial there of sheep that died of this disease, which is supposed to be the "very grievous murrain" referred to in Exodus—a view supported by the further statement that when Moses sprinkled toward heaven in the sight of Pharaoh handfuls of "ashes of the furnace," it became "small dust over all the land of Egypt, and "it became a boil breaking forth with blains (blisters) upon man and upon beast." This description would fit dried infectious material, which in the hot climate of Egypt could be readily cultured and dried. The biological warfare now being spoken of may have thus had a very early prototype. Anthrax (the word also means *carbuncle*) is known as splenic fever; in French it is *charbon*, a burning coal. It was also known as "wool-sorter's disease" because it was often carried to human beings by unsterilized wool and by animal hairs used for shaving brushes, etc.

In conclusion, it seems evident that the essential criteria of life are twofold: (1) the ability to direct chemical change by catalysis; (2) the ability to reproduce by autocatalysis. The ability to undergo heritable catalyst change is general, and is essential where there is competition between different types of living things, as has been the case in the evolution of plants and animals. Even with a single living unit, heritable catalyst change would be essential to permit survival in ever-changing vicissitudes of the milieu, including climate, irradiation, and chemical substances presented as foods or as poisons.

While we are most familiar with relatively complicated living units—from mites to elephants, from molds to oaks—we are confronted in genes, bacteriophages, mitochondria and viruses with units approaching the dimensions of large molecules or small mole-

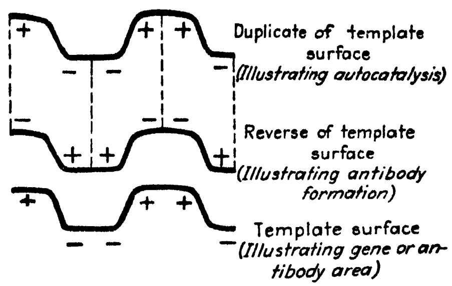

FIGURE 8a. Diagrammatic section of a specific surface, showing the complementary relation between reproductive catalysis (autocatalysis) and antibody formation. If conditions permit the formation and elution of a mono- or polymolecular layer or plaque of this nature, its subsequent activities depend upon maintenance of the integrity of its surface specificities. It could serve as the carrier or the prosthetic group for a biocatalyst (enzyme), or as a gene or antibody surface. In an editorial aroused by the paper of Alexander in *Protoplasma* (1931), Dr. Stephen Miall (*Chemistry and Industry*, London, Sept. 30th, 1932) first used the term *template* (also spelled *templet*) in this connection; but many others have since adopted this apt expression. (*See* page 4.)

cular groups. In some cases these might even be considered as molecules, or to use a term suggested by J. Alexander and C. B. Bridges[10] for the simplest conceivable unit, as *moleculobionts*. There is no evidence that anything below the level of the molecular has ever catalytically directed the formation of its like. The resting or latent forms of life seem to persist as long as the basic biocatalysts are not changed to so great a degree that resumption of active life has become impossible; and active life involves the direction of chemical change by the basic catalytic units, together with self-duplication (autocatalysis).

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Chapter 6

Catalysis: The Guide of Life

Two outstanding and superficially irreconcilable facts confront us when we consider the extremely complex and varied phenomena of life and of living units, or bionts. Most striking is the tendency of bionts to breed true. Despite wide differences in food and living conditions, the fundamental processes of life, growth, development and reproduction appear, as a rule, to be unerringly shepherded along definite paths in orderly sequences: overriding the vagaries of wind, wave and current, the ship of life tends to maintain a fixed course.

On the other hand, closer examination of the facts reveals that the course of life, like that of true love, never does run smooth. We have ample evidence of abnormalities in structure and function, some of which may be inherited. Sometimes these are helpful, but often they are harmful and lead to disease and death. No matter how we may differ in our attempts to explain these deviations from ideal normality, it is a fact that evolutionary changes occurred over pre-human geological epochs, that from the earliest times men have selected desirable spontaneous varieties of plants and animals for propagation. In passing, it may be mentioned that present scientific views as to the order of the appearance of living things are substantially the same as that given in Genesis: following the emergence of dry land came grass, herbs and trees; then living creatures of the seas, and birds; then living creatures of the earth, cattle and beasts; and finally, man.

In our attempt to explain the basic mechanism whereby life exists, persists and proceeds, we have invoked the old but now well known and extensively utilized principle of *catalysis*, which involves the direction of definite chemical changes by surfaces of definite structure under definite conditions. Naturally, many other mechanisms, such as differential diffusion, selective adsorption, and fluid movement are also operative, especially in relatively large and complex organisms, and at various structural levels. But

it is the highly specific surfaces found in such biocatalysts as genes, enzymes and similar areas, which direct the formation of the specific chemical molecules that underlie all larger structures and functions. Changes in biocatalysts or additions to their number, where they are persistent or heritable, result in changes in the biochemical output even under the same conditions; but the demonstrable consequences of these changes in what I may call the *chemism* or chemical output, may be so obscure and various that we are deceived as to their common basic origin.

It is believed that a wide range of biochemical and biological phenomena are readily understandable on the basis of specific catalysts, their formation and the changes which they undergo. No other simple, basic and ubiquitous mechanism is equally consonant with the extremely diverse experimental evidence over such a far-flung range, which includes immunology, genetics, embryology, medicine, evolution and paleontology.

What Is Catalysis?

A little over a century ago Jöns Jakob Berzelius, the great Swedish chemist and physician, coined the word "catalysis," and accurately anticipated modern views of its nature. Since then, it has been often erroneously taught that catalysts merely speed up reactions that would naturally occur of themselves in the course of "infinite time." Since *time is of the essence of all biological processes,* such a view, even if true, would be purely academic. Consider, therefore, the original statement of Berzelius.[1]

"It is then proved that several simple and compound bodies, soluble and insoluble, have the property of exercising on other bodies an action very different from chemical affinity. By means of this action they produce in these bodies decomposition of these elements and different recombinations of these same elements, to which they themselves remain indifferent.

"This new force, which was hitherto unknown, is common to organic and inorganic nature. I do not believe that it is a force quite independent of the electrochemical affinities of matter; I believe, on the contrary, that it is only a new manifestation of them, but since we cannot see their connection and mutual dependence, it will be more convenient to designate the force by a separate name. I will therefore call this force the catalytic force, and will call catalysis the decomposition of bodies by this force, in the same

way that one calls by the name analysis the decomposition of bodies by chemical affinity."

It is interesting to read the reaction of contemporaries of Berzelius to his views:[2]

"*On Catalysis and Catalytic Force, by Berzelius.* As is well known, in the action of gold, etc., on hydrogen peroxide, of acids on sugar, of sulfuric acid on alcohol, (according to Mitscherlich), of spongy platinum on hydrogen, etc., we have recently had examples, even though they are not all of like cogency, of the existence of various influences that substances exert on each other, which differ from heretofore known types of chemical activity. That is, there evidently are substances which possess the power to decompose into their constituents other materials with which they come into contact, without entering into combination to any appreciable extent with the new compounds formed. This type of chemical activity has been known as decomposition by mere contact. Berzelius proposes to call this power of substances *catalytic power*, and the process due to it *catalysis*. He does not mean to say that the force is a new one, but is convinced that we are here dealing with a special form of expression of known forces. Catalytic force seems to reside in the power of the substances in question to arouse in other substances, by their mere presence, affinities according to which the elements of the compound substance rearrange themselves, so as to attain complete electrochemical neutralization. This is analogous to the action of heat, and the question arises whether, as in the case of heat, a different degree of catalytic force will produce different catalyzed products from various substances, and whether different catalyzers will not produce different catalytic results with a given material. This cannot now be answered, for according to present experience, every substance having catalytic power appears to have it only as against certain other substances.[3]

Since many biological catalytic changes occur in the colloidal zone, the following excerpts from earlier publications are given, before considering some more recent aspects of catalysis. We begin with a fundamental paper of Thomas Graham:[4]

"The property of volatility, possessed in various degrees by so many substances, affords invaluable means of separation, as is seen in the ever-recurring processes of evaporation and distillation. So similar in character to volatility is the diffusive power possessed by all liquid substances that we may fairly reckon upon a class of analogous analytical resources to arise from it. The range also in diffusive mobility exhibited by different substances appears to be as wide as the scale of vapor tensions. Thus the hydrate of potash may be said to

possess double the velocity of diffusion of sulfate of potash, and sulfate of potash again double the velocity of sugar, alcohol, and sulfate of magnesia. But the substances named, belong all, as regards diffusion, to the more 'volatile' class. The comparatively 'fixed' class, as regards diffusion, is represented by a different order of chemical substances, marked out by the absence of the power to crystallize, which are slow in the extreme. Among the latter are hydrated silicic acid, hydrated alumina, and other metallic peroxides of the aluminous class, when they exist in the soluble form; with starch, dextrin, and the gums, caramel, tannin, albumen, gelatin, vegetable, and animal extractive matters.

"Low diffusibility is not the only property which the bodies last enumerated possess in common. They are distinguished by the gelatinous character of their hydrates. Although often largely soluble in water, they are held in solution by a most feeble force. They appear singularly inert in the capacity of acids and bases, and in all the ordinary chemical reactions. But, on the other hand, their peculiar physical aggregation with the chemical indifference referred to, appears to be required in substances that can intervene in the organic processes of life. The plastic elements of the animal body are found in this class. As gelatin appears to be its type, it is proposed to designate substances of the class as *colloids*, and to speak of their peculiar form of aggregation as the *colloidal condition of matter*. Opposed to the colloidal is the crystalline condition. Substances affecting the latter form will be classed as *crystalloids*. The distinction is no doubt one of intimate molecular constitution."

While it is impossible here to attempt to follow historically the development of colloid chemistry and of catalysis, the following brief quotation from a paper by Professor Leonard Thompson Troland of Harvard[5] clearly shows his appreciation of the problem:

"As a matter of fact, in the school of the physical chemists there has been in preparation, since the days of Thomas Graham, a system of knowledge which, even in its present unfinished form, has a most important and distinct bearing upon mooted biological problems. This is the science of the *colloidal state*. The difficult abstractions and elaborate classificatory scheme, in terms of which the theory is now stated, will tend to be cleared up as our study of colloids comes definitely under the dominion of the general electro-molecular theory of matter. Intimate contact with the latter has already been established, indeed, through recent remarkable contributions by Langmuir, dealing with the atomic consti-

tutions of solids and liquids. It is to colloidal chemistry that we must look for answers to the large majority of the fundamental problems of vital activity. These answers will be slow in appearing, however, if we refuse to look.

"In fairness, it must of course be admitted that may biologists are keenly alive to the importance of the theory of matter, and especially of the theory of colloids, for the advancement of their science. However, because the majority of these men are specialists in biochemistry, there seems to be a lack of coherent applications of modern physico-chemical ideas to the problems of evolution and heredity, which make up the heart of the biological mystery.

"It has for some years been my conviction that the conception of *enzyme action,* or *specific catalysis,* provides a definite, general solution for all of the fundamental biological enigmas: the mysteries of the origin of living matter, of the source of variations, of the mechanism of heredity and ontogeny, and of general organic regulation. In this conception I believe we can find a single, synthetic answer to many, if not all, of the broad outstanding problems of theoretical biology . . . It is an answer, moreover, which links these great biological phenomena directly with molecular physics, and perfects the unity not alone of biology, but of the whole system of physical science, by suggesting that what we call life is fundamentally a product of catalytic laws acting in colloidal systems of matter throughout long periods of geologic time. This view implies no absurd attempt to reduce every element of vital activity to enzyme action, but it does involve a reference of all such activity to some enzyme action, however distantly removed from present activity in time, or space, as a necessary first cause. Catalysis is essentially a determinative relationship, and *the enzyme theory of life,* as a general biological hypothesis, would claim that all intra-vital or 'hereditary' determination is, in last analysis, catalytic."[6]

In a paper by J. Alexander and C. B. Bridges[7] the following was stated: "Before considering the modern view of catalysis, let us first review some consequences of the structure of matter which make catalysis possible and specific. When electrons and protons combine to form atoms, when atoms combine to form molecules and when molecules combine to form larger groups, there are always left over some outwardly directed, unsatisfied fields of force. The residual

forces of molecules exert powerful attractions or repulsions on particles which come within the range of attraction.

"If we consider a single molecule, or a very small molecular group, *A*, it is obvious that the residual electrostatic or electromagnetic surface forces present to the milieu a mosaic which is highly specific in various directions. In Fig. 9 the specific nature of the surface of *A* is shown diagrammatically by the convention of specific jaggedness in the outline. In consequence of its mosaic pattern, a particle may exhibit several different kinds of specific actions separately or simultaneously, on different portions of its periphery.

"Let us now imagine a simple molecule, *B*, approaching *A*, and the process of fixing itself at the surface. This will occur only if the surface charges presented by the approaching molecule bear a lock-

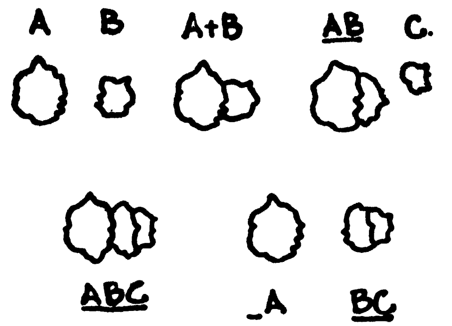


FIGURE 9

and-key relation to those of the particle, and if the velocity of approach lies within critical limits.

"As soon as the oppositely charged areas come within their critical distance, fixation occurs, as is diagramed $A+B$. Instantaneously thereafter occurs a mutual neutralization of forces and a complete reshuffling of all the internal and surface fields. The compound molecule, after the fixation, will present to the milieu a different configuration from what it presented before fixation. This is diagramed as *AB*. The former *B* portion might now be able to make other attachments previously not possible to it. The attachment of a second molecule, *C*, would once more cause a reshuffling of all the fields involved, as is diagramed *ABC*. Now, if the resulting bond between the two added molecules were stronger than the bond between the former *A* and *B* portions, then there might be released to the milieu a new type of duplex molecule, *BC*, while the fixation surface of *A* would be freed for renewed action.

"Suppose now that we have a catalyst particle composed of several simple molecular subunits which we can diagram roughly as *A* in Fig. 10. Suppose that at some one of its faces the catalyst fixes or absorbs

the several subunits of which it is composed; that, because of the order of their fixation or because of the reshuffling of electronic fields which follows each addition, these component subunits form a new group identical with the fixation or catalyst group, and suppose, lastly, that the duplicate particles now separate or are separated. Each would be an exact duplicate of the other in catalytic and in self-duplicating behavior. Our original particle could properly be called an *autocatalytic catalyst.*

"As an example of a simple and well-known chemical process, analogous in a rudimentary way to the autocatalytic synthesis postulated above, one may mention the formation of crystals of the alums. If to pure cold water is added the salt potassium sulfate and the salt aluminium sulfate, a solution strongly supersaturated for the potential double salt, alum, may be obtained. But, in the absence of a fragment of a previously formed crystal of alum the supersaturated

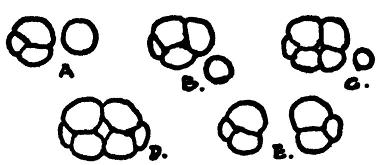


FIGURE 10

solution remains practically indefinitely without giving rise to alum crystals. However, if a tiny fragment of alum crystal is dropped in, or was present in the materials used, at its surface occurs an adsorption of the two separate salts together with a definite number of water units, the whole addition condensing into a very specific space lattice with repetitions of the unitary alum complex. Mechanical, or even thermal, agitation may break the bonds between the newly formed alum complexes and the parental crytsal, so that a host of descendants may grow and reproduce in the nutrient solution.

"We do not contend that an alum crystal constitutes simple life, although there may be some who would do so. In vital units the building stones of the finer structure are not potassium, aluminum and sulfanion, but are carbon, nitrogen, phosphorus, etc., which are united in a unit complex which is far more voluminous and involved than alum is. Besides this difference in constituents, or more probably because of it, the units properly called vital are more flexible in their bonding and more diversified in the activities carried on at the catalytic surfaces.

"The simplest living units of which we have indisputable evidence are the genes. The forces governing the reproduction of each gene are known to be *within* the individual gene. In evidence of this we need cite here only the well-known fact that in a heterozygote, each

gene of the allelomorphic pair picks out of the common milieu exactly those constituents which lead to its own particular formation. Furthermore, this specific formation occurs nowhere else in the cell except precisely at the locus occupied by each particular allelomorph. All these properties conform to our postulated autocatalytic reproduction.

"We would like to point out one more property of genes which has its analogue in a rudimentary way in the alum crystal. The growth rates of the genes of a given cell are remarkable in their uniformity. This holds true not only for a given temperature but throughout the great range of temperatures to which some organisms are adapted. If a particular gene did not keep step with the others it would either overreproduce or else fail to reproduce in time to be included in the daughter cells with the other genes.

"Not simply this uniformity in temperature characteristics, but a considerable number of other properties, for example, the universal synthesis of the substance called chromatin, which envelops the genes or the string of genes, and the remarkable similarity in chemical constitution of nuclear materials as revealed by the exceedingly crude methods of chemical analysis now available, all point to one striking conclusion. This conclusion is that the genes may all be viewed as simple variants of one fundamental structure. We may suppose that their power of autocatalytic synthesis is the outcome of the possession of a common pattern of structure, and that the different allelomorphs, or indeed the different genes in a given cell or even the different genes in apparently very widely separated forms, are the same except for differences in the fringe of the gene, in its side chains and in localized additions or substitutions.

"To return to our oversimplified analogy in the alum crystal: it is remarkable that alum crystals retain their specific structure through a host of substitutions of materials, or "mutations." Thus, rubidium, cesium and even ammonium can be substituted for the potassium; iron, manganese, etc., can be utilized instead of the aluminum; selanion (SeO_4), etc., instead of the sulfanion. The most essential characteristic of an alum crystal seems to be the particular strain set up in the local ether when alum crystals originated. It is the *structure,* the particular space-lattice present, that matters, and the points in that lattice can be taken by any materials whose outwardly directed fields of force are flexible enough to adapt themselves to the particular 'set' that is in the local ether."

The Industrial Importance of Catalysts

In past years, sulfuric acid, a basic material to chemical industry, was made in dilute state in large lead-lined "chambers" and

then concentrated, the oxidizing nitrous gases being recovered by "scrubbing" in Gay-Lussac and Glover towers. The sulfur dioxide was made largely from iron pyrites. This often contained arsenic which, unless removed at considerable cost, contaminated the acid and "poisoned" platinum catalysts, when these were used to oxidize the SO_2 into SO_3 in the presence of air. Due in part to the availability of cheap and pure sulfur brought up from our western "salt domes" by the Frasch process, most sulfuric acid is now made by passing a mixture of air and SO_2 over catalysts, and getting concentrated acid directly. Vanadium pentoxide is largely used as catalyst, since it is much cheaper than platinum to install, and is not so readily "poisoned."

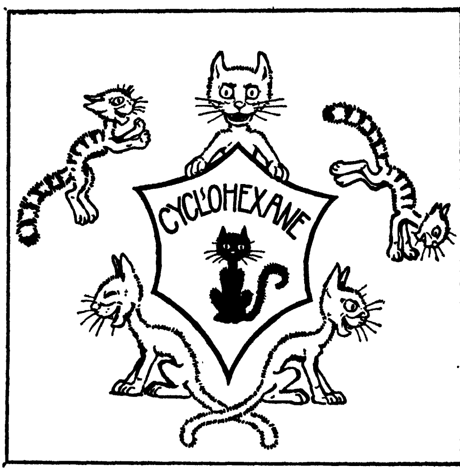


FIGURE 11. CATalysis. Design of a wooden medal for Kettering. Suggested to Baekeland, who did not believe cyclohexane could be manufactured.

During World War I, C. F. Kettering and Thomas Midgley, Jr., of the Research Department of General Motors, in the course of their collaboration with the U. S. Bureau of Mines to produce an aviation gasoline as free from "knock" as possible, made extensive engine tests with a variety of volatile organic liquids. The comparatively rare hydrocarbon cyclohexane was found to be a superior airplane engine fuel, and the suggestion was made that its manufacture be attempted. Dr. Leo H. Baekeland, then a member of the Naval Consulting Board, regarded the project as impractical and advised against it. He even promised a wooden medal to Kettering and his collaborators if they could make a single pint of cyclohexane.

Nothing daunted, the investigators applied to this problem their knowledge and skill in the preparation and use of catalysts for the hydrogenation of benzene and in a comparatively short time sent Dr. Baekeland a liter bottle of cyclohexane ensconsed in a plush-lined mahogany casket. Baekeland kept this on his desk for many years as a prized possession. And since the product had been made by CATalysis, Dr. Baekeland was furnished with a design for the wooden medal he had promised (Figure 11).

Today, catalysts and catalysis have achieved extensive publicity. The average newspaper reader, even if he does not know what a

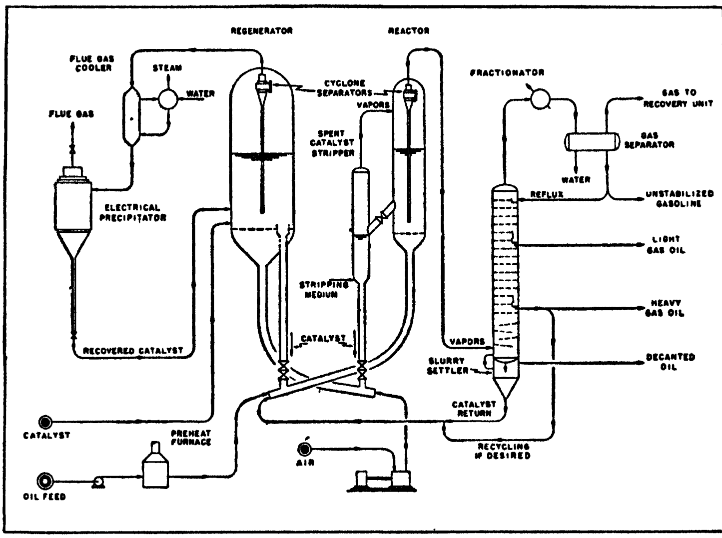


FIGURE 12. Flow sheet of a "cat-cracker" using the fluid catalyst process. (*Courtesy M. W. Kellogg Co.*)

catalyst is, does know that 100-octane gasoline, which gives our aviators speed and ceiling, is produced with the aid of catalysts. The more technically informed reader knows that the catalytic production of sulfuric acid, ammonia, nitric acid, and wood alcohol is an old story. In fact, Germany was able to start World War I in 1914 only because she had just then become independent of Chile as a source of nitrates, owing to German perfection of the Haber process, whereby nitrogen and hydrogen can be catalytically combined to produce ammonia. This, in turn, can be catalytically oxidized to nitric acid, the basis of most explosives as well as a vital ingredient in fertilizers. Those connected with the chemical industry or profession know that an ever-increasing num-

ber of useful and valuable organic compounds are now being catalytically produced, some in immense quantities. For example, the 1944 catalytic output of phthalic anhydride, used in making plastics, was over 62,000 tons. In 1936 about 250 tons of nickel were sold in the United States for catalytic use, about two-thirds

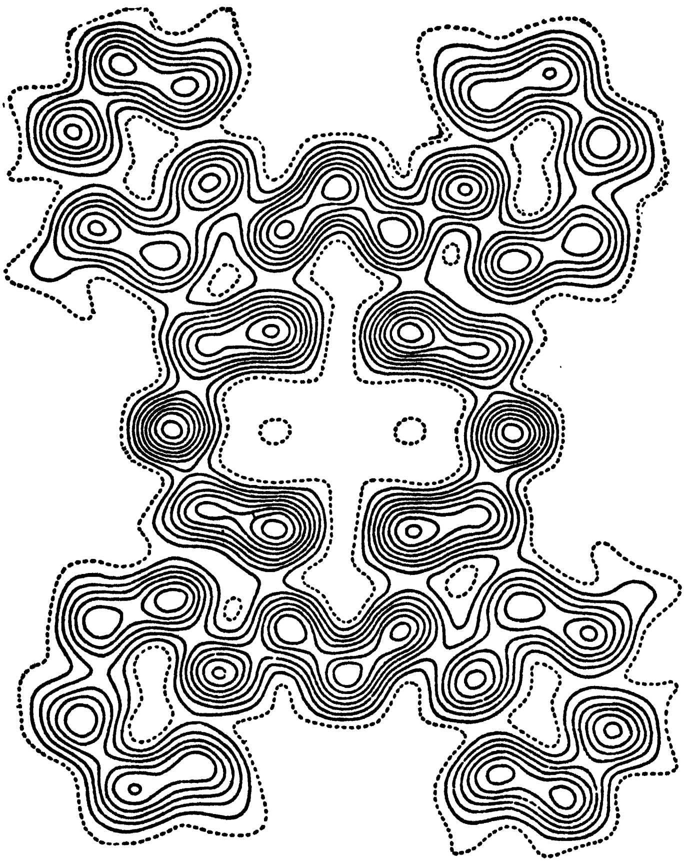


FIGURE 13. Projection of Phthalocyanine along the *b* axis, which makes an angle of 44.2 degrees with the molecular plane (*J. M. Robertson*). From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

having been used for hardening vegetable oils by catalytic hydrogenation to produce a huge tonnage of the well-known edible fats, which, like lard, are nonfluid at room temperature.

To illustrate the magnitude and importance of catalytic processes in the petroleum industry, consider in outline the recently perfected "fluid-catalyst" cracking process, devised by long and expensive cooperative research undertaken by large petroleum refiners, and now operating in about 30 plants (Fig. 12).

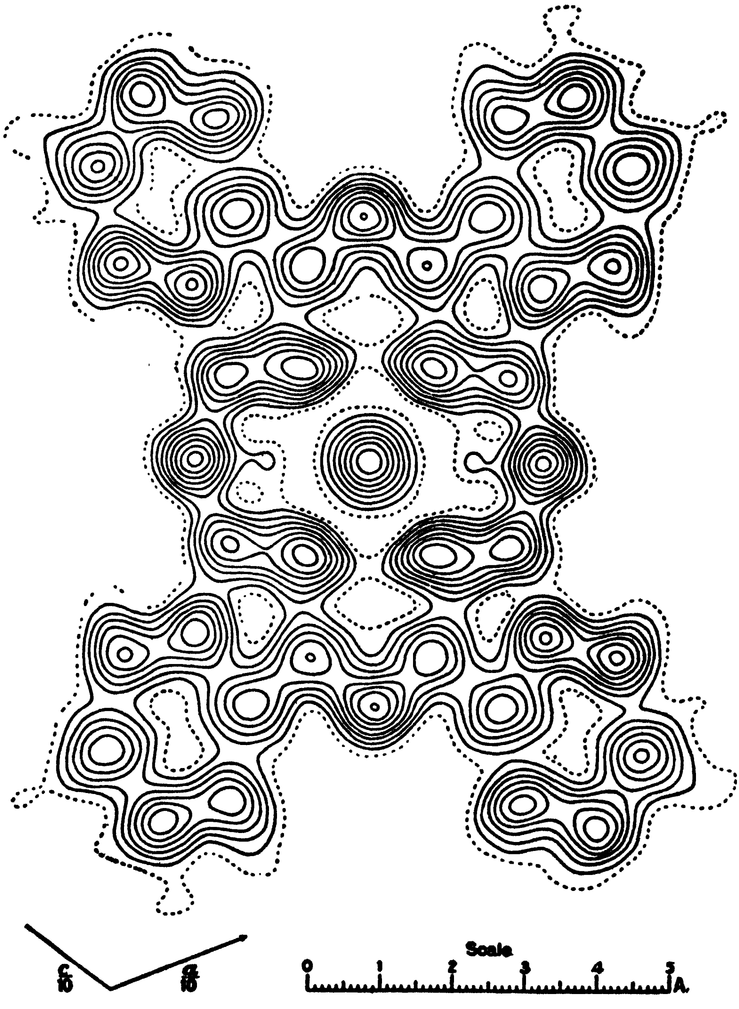


FIGURE 14. Projection of nickel phthalocyanine along the *b* axis, which makes an angle of 44.2° with the molecular plane (*J. M. Robertson*). Each contour line represents a density increment of one electron per A.², except the nickel atom where the increment is five electrons per A.² for each line. The one-electron line is dotted. (From paper in "Colloid Chemistry." Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

From a standpipe about 200 feet high, a claylike powdered catalyst cascades into a stream of vaporized oil at the rate of about 2 carloads a minute, and the torrid, oily duststorm swirls like a gas into a reactor vessel where, at the huge catalyst surface, there take place the complex chemical transformations termed "cracking." The cracked reaction products are separated from the now blackened, carbon-coated catalyst, which then falls into a stream of incoming air and is carried to a

regenerator where the carbon is burned off. The revivified catalyst is returned to the standpipe for reuse at the rate of about 40 tons a minute. About 73,000 tons of catalyst are consumed annually in this process, which has been yielding daily (after certain additions) over 400,000 barrels of 100-octane gasoline. Incidentally, there are also produced certain raw materials for synthetic rubber.

Germany developed the chemistry of coal and coal tar—because she had coal. American chemists realize that besides coal we have in our enormous supplies of natural gas and petroleum important raw materials for a great and novel organic chemical industry, in which catalysts are finding ever-increasing use.

Although our knowledge of specific catalysts and catalytic processes has been greatly extended by actual experiment, Professor P. H. Emmett states[8] that the selection of catalysts is still an art; the time has not yet come when it is possible to predict in advance with any degree of certainty the exact type of catalyst that will be most effective for a hitherto untried reaction.[9]

To illustrate the view of Berzelius that catalysis is "a new manifestation of the electrochemical affinities of matter," consider the electronic contour maps of phthalocyanine and of nickel phthalocyanine (Figures 13 and 14), as developed by Professor J. Monteath Robertson from x-ray spectrographic studies at the Royal Institution of London. Note how the introduction of a single nickel atom at the center changes the electronic contour or "physiognomy" at that point. The four benzene rings in the corners, as shown by the hexagons in Figure 15, are too far from the center to be greatly affected. The central core of phthalocyanine, represented by four pentagons in Figure 15, consists of four pyrrol groups, arranged similarly to those in porphyrin, a constituent of chlorophyll, cytochrome and other biocatalysts or enzymes. While the specific activity of chlorophyll depends upon its central atom of magnesium, hemoglobin and cytochrome have each an essential iron atom. A. H. Cook[10] found that of all the metallic derivatives of phthalocyanine tried, only the iron compound showed marked catalase activity; that is, it can catalyze the decomposition of hydrogen peroxide.

How Catalysts Function

Catalysts function by virtue of their outwardly directed specific fields of force, which produce characteristic distortions or warpings in the fields of susceptible particles (atoms, molecules, ions or larger particles) with which they come into contact for a sufficiently long time to permit a synthesis or a breakdown. The dis-

torted or "activated" particle may combine with other particles, or it may be split into smaller fragments. Catalysts thus function like a duly qualified judge, who may wed or divorce couples who come within his jurisdiction and remain there long enough for the operation of due process of law. Another analogy is that the catalyst functions like a "key" of a zipper closure. Drawn in one

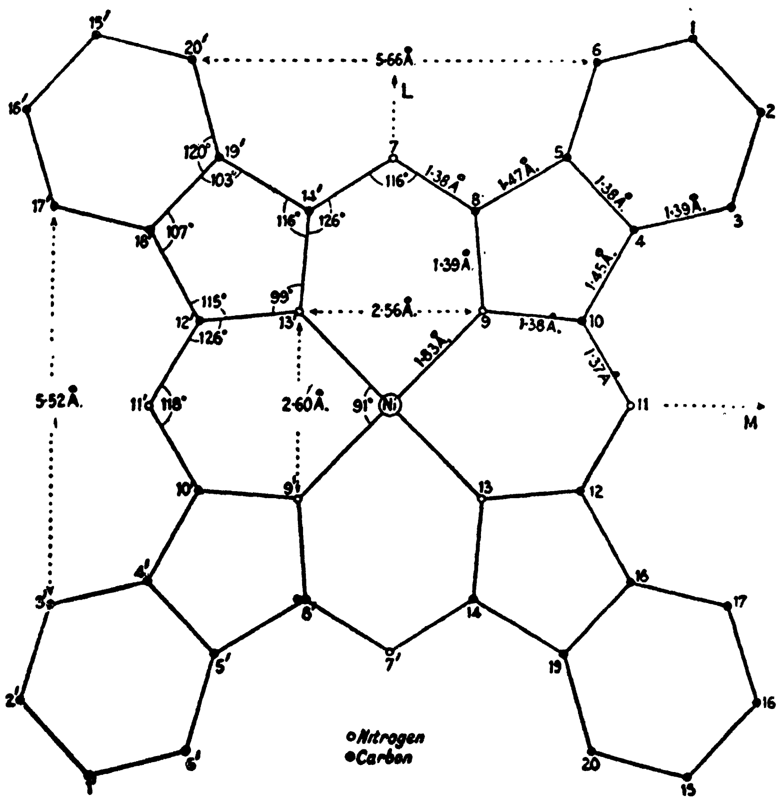


FIGURE 15. Dimensions of the nickel phthalocyanine molecule (*J. M. Robertson*). (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

direction, the "key" massages the opposing "hooks" so that they link together; if drawn in the opposite direction, the "key" releases the bonded hooks from each other, and the closure opens. The key and the hooks must be adjusted to each other within rather close limits of size and shape. An adhering bit of solder or paint, or a mechanical deformation of key or hooks may prevent normal functioning, but a single key may open or close an enormous number of bonds.

Similarly, a tiny quantity of a catalyst may determine the building up or the breaking down of an enormous number of molecules; but catalysts may be "poisoned" and rendered inoperative for certain reactions by physical or chemical change, or by adsorbed impurities. In developing what is known as the theory of active centers on catalytic surfaces, Professor Hugh S. Taylor of Princeton, in a paper sent to the Royal Society of London in 1925, discussed the phenomenon of progressive poisoning. Thus the surface of a nickel catalyst can be "progressively and successively poisoned for the hydrogenation of benzene with thiophene as a poison, then for the hydrogenation of ethylene which occurs on a surface whose activity for benzene hydrogenation has been destroyed by the poison used. A surface poisoned for both reactions could still serve for the reduction of nitrobenzene."[11]

It must be remembered that though the active catalyst areas may be but a small fraction of the total area of any particle or surface, what happens at this active area may have great and far-reaching consequences, *e.g.*, the production of a hormone, a vitamin, or the carrier or the prosthetic group of other catalysts. A tiny zipper key can open a very large bag. The importance of *local* happenings is also illustrated when a grindstone is used to sharpen steel tools. Without the use of water, oil or some similar lubricant, the local temperature at the metallic edge being ground may rise high enough to draw the temper of the steel. A dry grindstone immediately throws off a shower of sparks from steel. The frictional heat developed by a shoe brush melts the wax in the shoe polish, locally and momentarily. Sir George Beilby[12] showed that when metals are burnished or polished, molecular or atomic flow occurs; crystalline surface structure is practically fluidified and converted into an "amorphous" layer resembling a highly viscous liquid. Conduction dissipates the local heat developed.

It is common industrial practice to provide means for absorbing and removing the large amounts of heat locally developed by catalysts. For example, the vanadium pentoxide catalyst used to oxidize naphthalene vapor to phthalic anhydride in the presence of a limited supply of air, is kept at a safe operating temperature by insertion of iron tubes containing mercury, which boils at 357° C and thus prevents fusion of the catalyst or charring of the organic substances in process.[13] The various impurities in the naphthalene also suffer chemical or physical changes, but no investigation has been made to see if these are due to local heat, to specific action of the catalyst, or to both. In industry, the "waste heat" from the main reactor (where the main

reaction may be either endothermic or exothermic), is generally used to preheat reactants, to help initiate other processes, or to raise steam for general uses.

Temperature Control in Biocatalysis

Most living things die on exposure for varying times to the temperature of boiling water, especially in the presence of moisture. Some dry spores are more resistant. E. Zettnow reported[14] that some organisms in lime paste from a sugar factory survived heating for 30 minutes at 310-320° C. Subsequent tests reduced the killing temperature to 199° C and 220° C. In nature only those biological systems and structures survive which keep temperatures down to non-lethal levels, by protecting the delicate, heat-susceptible catalysts upon which life depends, against too high and too localized a liberation of heat. How is this accomplished in the cell?

Three main factors seem to be at work. (1) The over-all reaction is broken down into a series of successive chemical steps, mediated by different catalysts or catalyst areas, so that the whole reaction proceeds in what may be called a *step-wise* manner. (2) The intermediate products must move from one catalyst area to another (which takes time), and the total heat of the reaction is gradually set free at distributed areas in what may be called a *spot-wise* manner. (3) The biocatalysts are surrounded by aqueous cytoplasm; and water, with its high specific heat, helps to absorb and distribute the heat energy liberated.

The net result is that biological reactions usually proceed at a very *gradual* rate, without sudden and violent *local* temperature increase; this would be fatal, although the normal body temperature is maintained by the regulated liberation of heat. Many other factors are operative in control of body temperature, apart from orderly liberation of heat by the biocatalysts specific to the particular kind of animal. In cold-blooded animals (*poikilotherms*), the body temperature is much influenced by that of the surroundings. Migration or descent underground protects some against freezing to death, while others seek shade to prevent death from too long an exposure to the sun (*e.g.*, rattlesnakes). The temperature of warm-blooded animals (*homeotherms*) tends to remain constant, despite variations in the temperature of their surroundings; but it varies greatly with the position of the animal in the taxonomic scale.[15]

For protection against cold, warm-blooded animals have hair, fur, feathers, or layers of fat (whale). When we get "blue with the cold," our blood has stagnated to reduce heat loss; and shivering, probably initiated by the hypothalamus, represents involuntary "exercise." As protection against heat, animals seek shade, but rely mainly upon radiation of heat to the air, which may be aided by perspiration, since water has a high latent heat of vaporization. Dogs, which have no sweat glands, loll out their tongues, and I have seen sparrows near Merced, Cal., in the meager shade of fence posts, "panting" to endure a temperature which registered over 110° F in the shade above ground level.

Efficiency in Biocatalysis

The ideal heat-engine of Carnot (1824) has a theoretical efficiency of about 50 per cent. For steam engines, it is about 20 per cent, for gas engines about 25 per cent, for diesel engines about 40 per cent; but actual results may go considerably below these figures. A standard cadmium storage "battery" or cell, slowly charged and discharged, may approach 100 per cent efficiency; and it is to be expected that animals, operating likewise by chemical energy rather than by differences of temperature, will show high efficiency—and they do. But the situation is greatly complicated by the fact that animals utilize much of the energy of their food in mere maintenance and in growth and reproduction, which involve synthetic processes which require heat. The efficiency of some of these synthetic processes may be low, but the "waste" heat may be utilized in speeding up or initiating other chemical changes, as well as in keeping up the body temperature when distributed by the blood.

The efficiency of autotrophic bacteria in producing ammonia from atmospheric nitrogen approximates 3 per cent,[16] while that *Nitrobacter,* using the energy derived from oxidation of nitrite to nitrate, to assimilate carbon from carbon dioxide, is reported as about 6 per cent.[17] O. Rahn[18] estimates that one Calorie value in food produces the following heat liberation (in Calories) in various animals: pig, 0.2-0.4; trout, 0.18-0.31; cockroach, 0.34-0.35; mold, 0.58-0.70; colon bacillus, 0.13-0.24; pseudomonades, 0.21-0.22. Agriculturists estimate efficiency in terms of useful or marketable products produced—wool, meat, hide or skin, fat, eggs, milk, manure, etc.

By directing the course of chemical reactions with restricted heat emission, at localized areas and at regulated velocities, cata-

lysts may become sources of "quanta" of kinetic energy. Despite the influence of surrounding aqueous material, they may thus increase the intraparticulate activity or resonance of *some* molecules, or give them momentarily an increase in kinetic motion, corresponding *in effect* to what a relatively high temperature might do. A molecule so activated and accelerated could become highly reactive. In some cases, activation might be imparted to a specific portion of a large molecule or complex. These physicochemical aspects of the chemical changes which take place enable us to form some reasonable picture as to how the "driving" energy is distributed and utilized in what biological chemists term "coupled reactions."

In considering the significance of coupled reactions for the enzymic hydrolysis and synthesis of proteins, Drs. Max Bergmann and Joseph S. Fruton[19] point out that thermodynamic data alone tell us only the approximate amount of energy needed to make the system operative. "We must therefore look for the specific physical or chemical mechanisms which make possible the synthesis of peptide bonds." In certain reactions they discuss, sparingly soluble compounds formed crystallize out, and equilibrium conditions lead to further synthesis. "In these peptide syntheses, therefore, the energy required for peptide synthesis comes from the process of crystallization whereby the synthetic product is removed from the equilibrium." In these cases Bergmann and Fruton intimate that the kinetic energy (heat) set free by the aggregation (crystallization) of the synthesized product, is the mechanism whereby the energy is bandied about. The work of Prof. Rudolf Schoenheimer[20] "led him to the view that in the dynamic equilibrium between proteins and amino acids in the tissues, peptide bonds are constantly being broken and reformed under the catalytic influence of the tissue enzymes."

In the ordinary microscope, fat globules in highly diluted milk show an uneasy oscillation about a mean position. The ultramicroscope reveals the violent Brownian motion of the colloidal casein particles, whose swarming crowd can be seen jostling the fat globules this way and that. But the more rapid kinetic motion of the still smaller molecules of water and solutes is invisible, though it underlies what we see and is also responsible for bringing some molecules to a reactive state. Similarly, in the cell, the unseen molecular activity greatly exceeds the intense activity of particles made visible in the ultramicroscope.

Professor F. G. Donnan of the University of London concisely

summarized the work of A. V. Hill, W. Hartree, O. Meyerhof and others as follows:[21] "When the muscle tissue contracts and does work it derives the necessary free energy, not from oxidation, which is not quick enough, but from the rapid exothermic conversion of the carbohydrate glycogen into lactic acid. When the fatigued muscle recovers, it recharges its store of free energy; that is to say, by oxidizing or burning some of the carbohydrate, it reconverts the lactic acid into glycogen. Thus in the recovery stage we have the coupled reactions of exothermic oxidation and the endothermic conversion of lactic acid into glycogen."

In a fuller discussion, including more recent work, Professor Otto Meyerhof[22] observes: "Three different anaerobic reactions were found to be related to activity and also to be linked to one another: (1) splitting of carbohydrate, preformed in the muscle as glycogen and hexose-6-monophosphate, into lactic acid via phosphorylated intermediaries and pyruvic acid; (2) splitting of phosphocreatine into creatine and phosphate; (3) splitting of adenosine triphosphate to adenylic acid plus 2-phosphate via adenosine-diphosphate plus 1-phosphate." The lactic acid formation restores the phosphocreatine, broken down in the earlier stages of activity, while the breakdown of phosphocreatine is able to restore the adenosine triphosphate, and the oxidation of carbohydrate can reverse every cleavage reaction of (1), (2), and (3), whose respective energy stores are given as 1.2 Cals, 0.23 Cal, and 0.09 Cal respectively, whereas carbohydrate oxidation into CO_2+H_2O is listed as 30—60 Cals, and is, therefore, the main energy source. For many other important details and references, Meyerhof's paper must be consulted.

Summing up current views, Brody[23] points out that phosphate occupies a key position in biological oxidation. Pasteur observed its importance in 1860, Young and Harden confirmed it in 1905, and it is at present being extensively investigated by the Cori, Lipmann, and Meyerhof schools, among others.[24] Brody states: ". . . some phosphate esters serve as temporary biologic energy reservoirs, analogous to charged batteries. Thus, according to Cori, the synthesis of 6 molecules of glucose phosphate is coupled, or associated, with the oxidation of one molecule of glucose. A mol of glucose phosphate, therefore, has a labile energy increment which, depending on the energetic efficiency of the process, may be as high as 115 Cal ($\frac{1}{6}$ of about 700 Cal, the free energy of glucose). This is, presumably, what Lipmann refers to as phosphate-

ester bond energy, the main form or source of anaerobic energy as illustrated by the reaction:

$$\text{Glycerophosphate} + H_2O \leftrightharpoons \text{Glycerol} + \text{phosphate} + \text{energy}$$

. . . The phosphate group also catalyzes the oxidation and transport of fats. Other inorganic elements may participate in the oxidation of fat and perhaps its transformation into carbohydrate. Most electron donors (metabolites) must be phosphorylated as the preliminary step; for phosphorylation and oxidation are indeed *coupled* reactions."

Broadly, then, a "coupled" reaction is one so closely associated with another reaction as to determine the latter by providing the necessary energy. While it is conceivable that in some cases the total energy may be transferred without loss from one molecular grouping to another, it seems reasonable to believe that the kinetic aspects above outlined must generally be taken into account.

Furthermore, living things do not depend upon any one type of chemical change, the oxidation of glucose, though this is most important in so many plants and animals, including humans. The autotrophic bacteria utilize the energy available in some common exothermic reactions.[25] The "steaming" of manure piles and compost heaps is well known, and farmers sometimes use fresh manure as an "anti-freeze," as well as in hotbeds. Prof. J. M. Nelson of Columbia University informs me that in the early days in the Middle West, farmers often kept their sod-houses warm by a thick layer of fresh manure at the ground level.

Quite a number of biological reactions can take place in the dark; but in the synthesis of glucose by plants, solar energy is absorbed, with a local decrease in entropy. Animals are directly or indirectly dependent upon the glucose and other products synthesized by plants; in fact, most living things depend upon the catalyzed chemical output of others, and we are only beginning to understand these often curious and multiple relationships.

The Time Factor in Catalysis

The catalysts used in industry are sometimes aggregated colloids (*e.g.*, platinum sponge, nickel used in hydrogenation); but generally they are extended on carriers. This not only exposes a large active surface, but it also permits the finely divided catalyst to remain in place when gases or liquids are passed over it, and to be filtered off if it has been mixed into a batch.

Some biocatalysts are fixed, like genes in the chromosomes; but

most of the enzymes, which, with the genes, mainly direct the course of chemical change in bionts, are free particulate units of colloidal dimensions. The establishment of colloidal dimensions may come about in several ways. Some molecules are born colloids; some achieve colloidality by molecular growth or aggregation; and some have colloidality thrust upon them by adsorption or chemical fixation on a colloidal carrier or an extended surface.

Whatever the underlying cause, increase in particle size causes

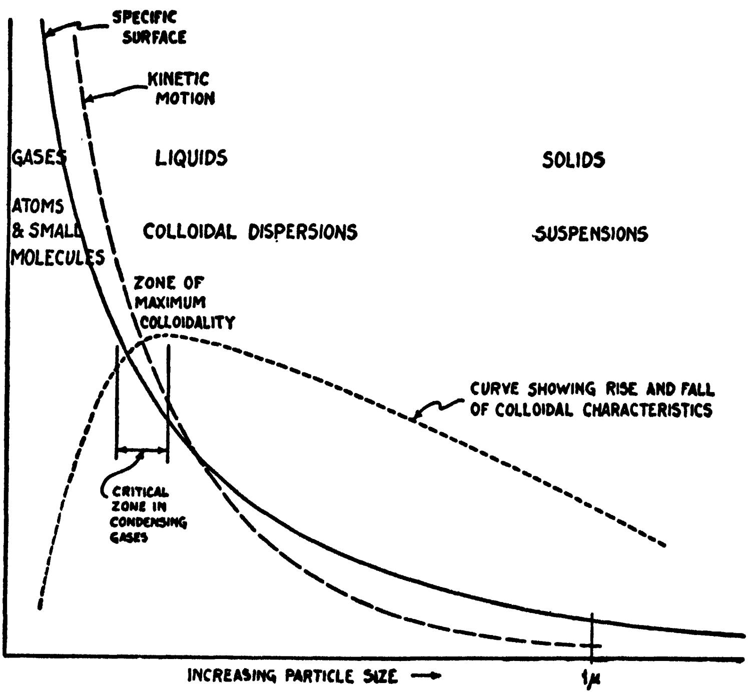


FIGURE 16. Degree of colloidality related to particle size, specific surface and kinetic activity. (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

a reduction in kinetic motion, as may be readily seen in an ultramicroscope. Particles at the lower ranges of microscopic resolution (about 0.25 micron) show only the feeble Brownian movement. On the other hand the kinetic motion of small molecules is so rapid that if a hydrogen molecule were large enough for the eye to resolve, it would still be invisible, just as in the case of a rifle bullet in flight. Between these two extremes lie all degrees of the kinetic motion of particles, which increases at an accelerated rate as molecular dimensions are approached.

But decrease in particle size is also accompanied by great increase in the area of surface exposed per unit weight. The degree of dispersion where this surface area exerts its most effective influence before rapidly increasing kinetic activity dominates has been termed the zone of maximum or optimum colloidality.[26] Figure 16 is a *diagrammatic* sketch of these relationships, based on the *assumption* that the particles are spheres, which, of course, is seldom the case. Particles have various, and even varying contours (threads, rods, plates, aggregates); the diagram simply illustrates the principle.

When a particle approaches an active catalyst area, two factors are of outstanding importance:

(1) the momentary electronic contours of the surfaces in apposition, for this determines the *possibility* of particulate union or influence;

(2) the kinetic velocities of translation and rotation of moveable areas, which determine whether the possibility may become a *reality*.

The importance of this kinetic factor came to the fore in the course of a lecture experiment made by Sir Ernest Rutherford at the Toronto meeting of the British Association for the Advancement of Science (1924). It was designed to illustrate how a positively charged atomic nucleus will repel an alpha particle emitted by radium. An electromagnet, with its positive pole up, was fastened to a table so that another electromagnet, swinging from the ceiling with its positive pole down, could just pass over it. The mutual repulsion of like magnetic poles caused the swinging magnet to take a parabolic path in any *off-center* approach. To illustrate the relatively rare recoil which occurs when an alpha particle makes a *direct* approach to an atomic nucleus, Sir Ernest took careful aim—but the swinging magnet passed completely over and beyond the fixed one, probably giving a slight unseen "jump" as it did so. Quickly retrieving the swinging magnet, he swung it again from a lesser distance and got the expected recoil.

From this it is evident that if a certain relative critical velocity is exceeded, particles or surfaces having the power to cohere, will *not* do so. In catalysis, a certain period of *time* is necessary for the electronic fields of the reactants to become fitted to each other, so that apart from the effects of rotation of the particles, internal kinetics, and mode of presentation, the kinetic velocity of translation is an important factor.

Increase in thermal agitation increases the *total number* of encounters *per unit of time* between reactant catalyst and substrate areas, and this works in favor of increasing the number of *fruitful* encounters. As the temperature increases, however, a point is reached where so many particles have so high a relative velocity that the number of *unfruitful* encounters increases to such an extent that the catalyst efficiency per unit of time *tends* to fall off.

Many other intercurrent factors may influence the thermal optimum in catalysis besides the kinetic factors; *e.g.*, the catalyst, reactants, or end products may undergo decomposition; adsorbed substances may "lame" the catalyst; specific substances or ions may affect the degree of dispersion or electronic integrity of catalyst or reactants. Thus Svedberg found that many proteins are dispersed into smaller subunits by change in pH, some of them being reconstituted from the fragments when the pH is shifted to the stability zone; and many substances will "dissolve" or disperse glue at room temperature, *e.g.*, calcium chloride, sodium nitrate, sodium naphthalene sulfonate.

Pepsin is "activated" by hydrochloric acid. What this means kinetically may in part be followed ultramicroscopically. A dilute solution of egg white heated nearly to boiling gave an opalescent dispersion full of bright, rapidly moving ultra-microns. On allowing a droplet of pepsin solution (Fairchild's containing 15 per cent alcohol) to diffuse under the cover glass of the slide, the albumin ultramicrons immediately coagulated into large, motionless masses. When a droplet of 0.1*N* hydrochloric acid was introduced under the cover glass the coagulated masses burst into small groups and isolated ultramicrons, which resumed their active kinetic dance. But almost immediately the albumin particles began to grow fainter and to disappear, the field meanwhile becoming brighter as smaller ultramicrons or amicrons were formed. The addition of pepsin to the opalescent albumin solution caused it to clear gradually at room temperature.

The kinetic motion of diastase particles may likewise be followed ultramicroscopically, as they gather about and actively rub or "gnaw" holes or cavities in starch granules. The chemical changes occurring are, of course, far below the level of visibility.

Haurowitz and Schwerin[27] found that the catalytic auto-oxidation of linoleic acid by hemin in *heterogeneous* emulsions ceases when the system is made *homogeneous* by addition of acetic acid, dioxane, pyridine, alkali or bile, but recommences when homogeneity is abolished by addition of water or stronger acid. These authors believe that this reversal demonstrates that the catalysis takes place only in the interfacial film between the water and oil phases, and attribute the observed diminution of the velocity of oxidation by

excess of hemin to displacement of linoleic acid molecules from the interfacial film. They conclude that orientation of substrate and hemin molecules in the interfacial film is of decisive importance for the establishment of the catalysis. This is, of course, true, and it involves the electronic contour factor (1) mentioned above; but the kinetic factor (2) is also to be reckoned with.

How Catalysts are Modified

The technological and patent literature has long reflected the fact that the introduction of new atoms or molecules into the active surface of a catalyst may stimulate, inhibit, or alter the nature and/or rate of the catalyzed reaction.[28] If a desired result is accelerated, the added substance is termed a promotor; if it is inhibited, the added substance is called an inhibitor or poison. In cases where a catalyst can direct the formation of several substances and an inhibitor is found which depresses undesired reactions, we speak of "beneficial poisoning." In a general sense any substance which changes the behavior of a catalyst may be called a modifier, a term free from implication as to the result of the change. The importance of *catalyst modification* in biology will be referred to later on.

One factor in the efficiency of a catalyst is the amount of free active surface it exposes to the reactants. It is common commercial practice to distribute the catalyst substance on carriers, or supports, such as asbestos, charcoal, alumina, diatomaceous earth (kieselguhr), etc., which are supposed to act merely mechanically. It has been found, however, that the carrier often exercises a marked effect, either by contributing small amounts of impurities to the catalyst surface (which may thus be either promoted or poisoned), or else by forming with the catalyst molecules some new composite surface of different activity.[29]

According to recently released information[30] a substantial amount of the synthetic rubber produced during the recent war was made from ethyl alcohol by the Lebedev process. An efficiency of about 65 per cent in butadiene synthesis was obtained with a silica gel catalyst promoted by tantalum oxide, and nearly as good results were had with the more plentiful zirconium oxide as promotor.

The potent effect of catalyst poisons is brought out by the remarks of H. Bernthsen at the Eighth International Congress of Applied Chem-

istry (1912) relative to the iron catalyst used in the first step of the Haber process:

"Extremely minute quantities of these bodies (impurities), which are almost always present even in the purest commercial products or in so-called pure gases, suffice to render the catalysts absolutely inactive or at least to diminish their activity very seriously. Thus iron, for example, prepared from ordinary iron oxide with a content of one per thousand of sodium sulphate is, as a rule, inactive. Iron containing 0.1 per cent sulphur is generally quite useless, and even with 0.01 per cent is of very little use, although in appearance and when examined with the ordinary physical and chemical methods no difference at all can be detected as compared with pure iron.

"The recognition of these facts gave rise to two problems: (A) The preparation of contact masses free from poisons or the removal of poisons from them; and (B) freeing the gases to be acted upon catalytically from all contact poisons. A trace of sulphur, one part per million, in the gas mixture, can under certain conditions be injurious, so that electrolytically prepared hydrogen must generally be further specially purified."

Since a small amount of a catalyst may, if given sufficient time, direct an enormous amount of chemical change, we can understand how minute may be the quantity of promotor or modifier needed to produce a great change in the final output.

Experimenting with the catalytic production of methanol from carbon monoxide and hydrogen, Sir G. T. Morgan[31] observed that while catalysts made by calcining equimolecular mixtures of manganese and chromium nitrates gave methanol containing only traces of higher alcohols, catalysts prepared by precipitating a mixed solution of oxides of manganese and chromium in caustic potash gave a product containing appreciable percentages of higher alcohols. This led him to try the effect of regulated additions of alkali metals to the catalyst. To quote one outstanding case, when the catalyst contained 15 per cent of rubidium hydroxide, the carbon in the gases passing over the catalyst was distributed as follows: (with equimolecular proportions of the pure Mn and Cr oxides the yield of methanol was 80.5 per cent).

Methanol	41.5%
Ethanol	1.6
Higher alcohols	36.7
Aldehydes, acetals, ketones	15.5
Methane	2.0
Carbon dioxide	2.0

The higher alcohols in this case consisted chiefly of isobutanol, but contained besides normal propanol, 2-methylbutanol, 2-methylpentanol, and 2, 4-dimethylpentanol.

Morgan found that the addition of cobalt to the catalyst gave mainly straight-chain alcohols, rather than the branching-chain alcohols formed by alkalized catalysts. This is shown by the following table which gives in *parts per thousand,* the alcoholic content of the *liquid* formed by passing the same mixture of carbon monoxide and hydrogen over different catalysts, under like conditions of temperature and pressure.

Alcohols Formed	Catalyst Used					
	Rb-Cr-Mn		Co-Mn-Zn-K		Co-Cu-Mn	
Methyl	420	—	198	—	220	
Ethyl	12	—	86	—	200	
n-Propyl	43	—	17	—	50	
iso-Butyl		69	—	11	—	3
n-Butyl	—		4	—	16	
β-Methylbutyl	—	8	—	1.5	—	2
n-Amyl	—		1		6	
β-Methylamyl	—	6.5		—		—
n-Hexyl	—		—		2	—
n-Heptyl	—		—		1	—
Unidentified	89		1.5			

In concluding his address before the Société Chimique de Belgique[32] Morgan said:

"All these experiments are of special significance, for they permit us to see the enormous variety of organic compounds which can be synthesized by secondary reactions following primary condensation of carbon monoxide and hydrogen, a gaseous mixture well known under the name of 'water gas,' made on a large scale from coal and steam.

"Up to the present time these condensations have been studied at temperatures higher than the decomposition of many organic compounds. With higher pressures and more efficient catalysts, we may some day expect to lower the reaction temperature very materially. When we shall have reached this point, the synthesis of organic compounds of great complexity should be possible and among these products we will find organic substances which up to now can be formed only by the chemical activity of living beings."

Biocatalysts; Prosthetic Groups; Symplexes

The course of chemical change in organisms is directed by catalyst units of colloidal dimensions—enzymes and genes, and perhaps also by units in some symbionts, and units fixed at or on various surfaces. Whereas with primal bionts gene-like units were probably the sole directors of life chemistry, in higher forms we find thread-like aggregations of genes (chromosomes) and a great

variety of secondary but highly specific catalysts (enzymes), whose production is in some way interwoven with the activities of the truly living, self-reproducing catalysts. The experimental evidence indicates that large numbers of biocatalyst units are destroyed or inactivated in the course of their functioning, so that there must be a continual replenishment of the supply.

How do biocatalysts arise? The broadest, and therefore the most indefinite answer to this question would be: by the assemblage of smaller units (atoms, molecules, colloidal particles) so as to constitute an exposed surface which has a suitable electronic structure and contour. Genes in the chromosome string thus form duplicates of themselves (autocatalysis), and must therefore be considered as living. On the other hand, an active catalyst area may be formed by being built into or upon a larger fixed surface, *e.g.*, a cell wall; or else there may be formed by adsorption and/or chemical combination, colloidally dispersed units—the multitudinous enzymes—which can flow and kinetically "swim" about in cytoplasm, sap, or body fluids, and may even diffuse slowly. Texts often give the erroneous impression that colloids will not diffuse; but Thomas Graham[33] clearly pointed out that they may diffuse, though they "are slow in the extreme."

The catalytic effect of the walls of the containers in which chemical reactions are carried out is not, as a rule, sufficiently appreciated, and traces of substances existing in, forming on, or introduced into these surfaces may powerfully influence results. Thus a food product processed in aluminum vessels was deleteriously affected by tiny fragments of iron left in the aluminum surfaces when these were scoured with steel wool. G. Bredig[34] found that by fixing amino groups to cellulose, wool, or silk fibers, organic catalysts were produced which split off carbon dioxide from bromcamphocarbonic acid. Although inorganic catalysts may appear to be simple, *e.g.*, platinum black, those having first-hand experience with commercial catalysis know how potent is the influence of structure, carriers, and impurities on their functioning.

Biocatalysts are composed mainly of complex organic molecules, assembled into a delicate but specific structure. When a certain molecular group enters a biocatalyst to form and characterize the active catalyst area, it is termed a *prosthetic* group. The word "prosthetic" is derived from a Greek root meaning to *add to*, or *to insert*. Thus prosthetic dentistry deals with the insertion of missing teeth. The prosthetic group, so to say, puts "teeth" into

the biocatalyst. Often the prosthetic group demands for its functioning the presence of a single atom, *e.g.*, magnesium with chlorophyll, copper in the polyphenol oxidase of mushrooms and in tyrosinase, iron in catalase, cytochrome, etc. Where "homeopathic" doses are effective, their action may often be understood along these lines.

Richard Willstätter[35] proposed the term *symplex* for compounds where high-molecular substances are bound by residual valencies, *e.g.*, a prosthetic group and a colloidal carrier. Symplexes are distinguished from mere mechanical mixtures by one or more of the following characteristics: (1) alteration or enhancement of specific reactivity of one component; (2) change in solubility or dispersion of one component; (3) change in optical properties; (4) change in stability; (5) change in toxicity; (6) change in reactions, *e.g.*, color reactions.

True chemical combination is not necessary to make separate units acquire new properties when combined. Neither an arrowhead, an arrow shaft, nor a goose feather is an arrow; but the three, when properly fitted together, will make an arrow and will function as one.[36]

Structures of the "symplex" type are very loosely bound together. E. L. Smith[37] found that solutions of phyllochlorins (chlorophyll-protein compounds extracted from spinach leaves) may be split into free chlorophyll and protein by the detergents sodium desoxycholate, bile salts (mainly sodium glycocholate) and digitonin. In the presence of sodium dodecyl sulfate (SDS), the prosthetic group remains attached to the protein, but the compound is split into smaller units, the protein properties and absorption spectrum being modified. Tobacco mosaic virus is also split by SDS into smaller fragments, nucleic acid being simultaneously separated from the protein.[38] In the presence of SDS chlorophyll loses magnesium and becomes phaephytin; and this substance, or the chlorophyll (depending on pH), remains attached to the protein, since the prosthetic group is not separated by ultra-filtration, dialysis, or fractional precipitation. Smith believes that much previous work on chlorophyll dealt only with the prosthetic groups of extremely complex specific catalysts. Emerson and Arnold[39] concluded from photochemical studies that 2,500 chlorophyll molecules form one functional unit in photosynthesis.

The importance of the carrier in the biological field is indicated by the following two examples. Keilin and Mann[40] report that peroxidase, which shows great resemblance to methemoglobin, can be considered as a compound of protohematin with a native protein.

Peroxidase forms two highly unstable compounds with H_2O_2, whose decomposition is much accelerated by the acceptor present in plant extract or by the addition of other acceptors, *e.g.*, ascorbic acid, hydroquinone, or pyrogallol. "The same hematin nucleus combined with three different native proteins forms three distinct compounds: methemoglobin, catalase, and peroxidase, which have many properties in common but show, however, striking differences in the nature and magnitude of their catalytic activities." Warburg and Christian[41] report that the old "yellow enzyme" may really be an artifact resulting from the loss of adenylic acid during its preparation. They describe five different yellow enzymes, some with similar proteins but different prosthetic groups, others with the same prosthetic group but different proteins. The protein/prosthetic group combination is reversible, and it is estimated that one molecule of alloxine dinucleotide can transfer 1440 molecules of oxygen per minute. Vegetables and fruits are "blanched" by steam before being dehydrated, to inactivate enzymes which cause undesirable changes.

This gives us some insight as to the potency and flexibility of the enzymic catalyst systems of living cells, and effectively answers the antiquated gibe of orthodox organic chemists that whenever a biological reaction is to be explained, a new enzyme is invented.

The formation of chlorophyll seems to demand the presence of iron, just as the formation of erythrocytes in man demands the presence of copper. The mode of formation of prosthetic groups is generally obscure,[42] but it has recently been shown that thiamin (vitamin B_1) is combined with pyrophosphoric acid,[43] and D. E. Green states:[42] "There is ample evidence of the existence in animal tissues and microorganisms of enzymes which catalyze the phosphorylation of thiamin as well as the dephosphorylation of diphosphothiamin." Some animals (man) must eat vitamin C; others (rats) can synthesize it, or in any event do without it in their food.

Nature, with infinite time and opportunity for experiment, accomplishes results in manifold and devious ways. Thus the ascidian *Phallusia mamillata* has an acid blood (3 per cent H_2SO_4) which contains as chromogen a non-dialysable organo-vanadium compound. On plasmolysis this gives a brown solution, which yields, on drying, a dark-blue powder showing over 10 per cent vanadium (two analyses gave 10.36 and 15.4 per cent, but the latter figure is doubtful). Mussels have a manganese-containing catalyst, pinnaglobin. Most crustaceans, including *Limulus* (the horse-shoe or king crab, of antediluvian ancestry) are literally blue-blooded because of copper-containing hemocyanin. A group of African birds known as the Touracous or Plantain-eaters have in their pinion feathers red or crimson patches or portions from which weak alkali or soap solutions will extract a pigment, which, after precipitation by acid, dries to a rich crimson

solid[44] called turacin. This has been recently proven to be a copper porphyrin compound.[45] Over sixty years ago Professor James F. W. Johnston (University of Durham, England) made the following comment:[46]

"The existence of an animal pigment so rich in copper as turacin (about 8 per cent), offers many interesting problems for study. Traces of this metal seem generally diffused in most vegetables and many animals; but here are more than traces—weighable and visible quantities. It is true that these plantain-eaters have been seen to pick up in their native countries grains of malachite, the green mineral carbonate of copper; but we must rather look to the vegetable food they consume as the true source of this metal. And when the copper is ingested, how does it find its way, in the complex pigment of which it is an essential part, precisely to those feathers, and to those barbs of feathers, and to those parts of such barbs, which are red, and not to the black portions? For if one of these feathers is burnt in a Bunsen gas-burner, not till the red part of the feather is reached will the green flash of the copper tinge the flame. However, in the *crest* of the violet plantain-eater (*Musaphaga violacea*) and perhaps traces in blood of all these birds, turacin, and therefore copper, does occur. Still the whole mystery of this strange pigment is far from being understood."

It has recently been reported that certain molds will not grow in the absence of traces of gallium; and Aguilhon and Sazerac[47] found that the addition of 0.0001 per cent of uranium acetate to a fermentation mixture of *Acetobacter suboxidans* and sorbitol increased the yield of sorbose by 76 per cent.

In an article on the biochemistry of microörganisms C. B. Van Niel[48] refers to many other cases where trace substances are vital: *e.g.*, molybdenum for the nitrogen-fixing organism *Azobacter*; copper for common molds as well as for higher plants; boron, apparently needed to form borocitrin, related to the flavin pigments and found in microorganisms by Kuhn. Van Niel also tabulates the recent work of various investigators showing the diverse ways in which different organisms split acetate and normal butyrate.

E. C. Auchter,[49] Chief of the Bureau of Plant Industry, U. S. Dept. of Agriculture, in discussing the interrelation of soils and plant, animal and human nutrition, lists some of the "physiological troubles" of plants which can be cured by supplying the necessary small amounts of certain missing but essential elements. It must, of course, be remembered that larger amounts than the tiny optima may be very harmful, as is the case, *e.g.*, with boron. *Magnesium* cures sand drown of tobacco in the soils of the coastal plain; *manganese* cures chlorosis of tomatoes on some calcareous Florida soils, and permits such soils to give improved yields of potatoes, snapbeans, cabbage, lettuce, peppers, carrots, beets, citrus and corn; *zinc* cures pecan rosette in the South,

citrus leaf mottle in California, South Africa, and Florida, little leaf of apples and other deciduous fruits in the West and Northwest, and white bud of corn on the Norfolk and Hernando fine sands of Florida; *boron* cures internal browning of cauliflower and dry rot of sugar beets in Michigan, crown rot of sugar beets in Ireland, die-back of citrus in Africa, internal cork and drought spot of apples in British Columbia, West Virginia and elsewhere, and cracked stem of celery in Florida; *copper*, added to muck soils in Holland and parts of western New York and to organic soils of Florida, improves the growth of several crops; *sulfur* corrects yellows of tea in Nyasaland (Africa), and improves the growth of field crops in Oregon and other areas.

Since animals depend upon plants, "the soil is the mother of all living things." Deficiency of cobalt[50] in certain New Zealand soils causes "bush sickness" in sheep, which seems identical with the "pine" of sheep in Scotland. Iodine deficiency leads to goiter in man and beast and caused an annual loss of thousands of pigs in Montana until iodine feeding was practiced. Selenium, taken from the soil by plants, causes disease and malformations in animals eating the plants.[51] Marco Polo, on his journey to Tartary, observed hoof deformations of this type, which may have had the same kind of origin. According to A. L. Moxon traces of arsenic compounds, fed in water or in salt, tend to protect animals against the toxic action of selenium, while bromobenzene aids in its excretion.

The eighth scientific meeting of the Nutrition Society held in London, England, Oct. 17th, 1942, was devoted to "Trace Elements in Relation to Health." Among the rarer deficiency diseases mentioned were: enzootic ataxia (swayback of lambs), which may be prevented by feeding traces of copper during pregnancy; molybdenosis (called teart of Somerset), which affects ruminants, due to molybdenum taken up by plants where the soil content of molybdenum was about 100 ppm, and helped by traces of copper sulfate; fluorosis, which may cause osteosclerosis, though teeth with mottled enamel due to fluorine are relatively immune to decay.

It must not be supposed that trace substances always operate in one way, for the complications in any case may be great. Thus sulphur may be oxidized by soil bacteria and affect the local pH—from which a chain of other consequences may follow. However, much of the evidence points to the conclusion that the inclusion of trace substances in catalyst surfaces is a frequent factor that must always be considered; for visible results develop from the products of catalysis.

Chemical Activators

A great mass of evidence has accumulated to indicate that many important biological changes are directed, or powerfully influ-

enced, by *exceedingly minute proportions* of highly specific substances, even though the *actual number of molecules* (or other particulate units) involved may be enormous. Some activators come from outside the organism, *e.g.*, vitamins from food, trace substances from the soil; others are formed within the organism, *e.g.*, auxins in plants, hormones and neurohumors in animals.

Electric phenomena are associated with the functioning of nervous and muscular tissue. Electrocardiograms from the heart muscle and electroencephalograms from the brain are routine precedures in many hospitals. Sir Edgar A. Adrian (Nobel prize, 1932) and his collaborators measured the tiny electric impulses passing along nerves.[52] Herbert S. Gasser and Joseph Erlanger (Nobel prize jointly, 1944) applied the oscillograph to demonstrate the details of these impulses. Based on interpretations of experimental work of Elliot, Howells, Dixon, Otto Loewi (Nobel prize, 1936), W. A. Cannon and Sir H. H. Dale (Nobel prize, 1936), it has for many years been thought that nerves act upon effector organs (*e.g.*, muscles and other nerve cells) by releasing at the nerve endings chemical substances (*e.g.*, acetylcholine, sympathin, adrenaline) which exert a stimulating or an inhibiting effect at their place of liberation. These views were based on experiments showing the *pharmacological* effects of acetylcholine, and a demonstration of its presence, under certain conditions, in the perfusion fluid after stimulation.

The new concept is based on studies of the enzymic catalysts which form and break down acetylcholine, and on the correlation of these reactions with the physical happenings *in the living cell.* Quite a variety of recent experimental data indicate that the propagating agent along nerves and muscle fibers, as well as across nerve synapses and neuromuscular junctions, is a flow of current —the action potential. However, acetylcholine plays an essential role in the alteration of the surface membrane (the "progressive disturbance" of Keith Lucas), permitting the flow of current. The following abstract was kindly prepared for me by Professor David Nachmansohn of Columbia University, who has done so much to establish the new view.

"The enzyme which inactivates acetylcholine by splitting the active ester into its inactive compounds, choline and acetate, is cholinesterase. One of the most essential features resulting from studies of this enzyme is the high speed with which acetylcholine may be inactivated. This speed parallels that of the electrical manifestations,

a prerequisite for any theory correlating chemical reactions with conduction. Significant amounts of the ester can be metabolized in a millisecond or a fraction thereof. One square millimeter of neuronal surface of the giant axon of Squid, *e.g.*, can split one billion molecules of acetylcholine in one millisecond. The turnover number of the purified enzyme seems to be close to 20 million per minute, which means that one molecule of enzyme can split one molecule of substrate in a few millionths of a second. The enzyme is localized exclusively in the suface where the bioelectrical phenomena occur. It is present in all conducting mechanisms throughout the whole animal kingdom, being found in the lowest animal form possessing neuromuscular tissue (Tubularia, a hydrozoan coelenterate). The enzyme is distinctly different from all esterases present in non-conductive tissues like liver, kidney, pancreas, etc.[53]

"The activity of this enzyme can be correlated in different ways with the electrical manifestations of nerve activity. A close parallelism between voltage and cholinesterase activity has been established in studies on electric tissue. The powerful discharge in these organs is basically identical with the electric potentials produced in nerve and muscle, the only distinction being the arrangement of the units in series. Great variations of the voltage per centimeter are found in the electric organ of *Electrophorus electricus*, the South American electric eel, whether the organ of a single specimen or that of specimens of different sizes are used. In experiments in which the voltage ranging from 0.5 to 22 per centimeter was plotted against cholinesterase activity, a direct proportionality was found between the physical and the chemical event, the line correlating the two processes passing through zero. These findings indicate the interdependence of the two events. No other chemical reaction offers a comparable behavior.[54]

"Another line of observation was based on thermodynamic considerations. If the primary alterations of the surface membrane are connected with the release and removal of acetylcholine, then the primary source of energy during recovery should be used for the resynthesis of the ester. In experiments on the electric fish, it can be demonstrated that energy-rich phosphate bonds are adequate to account for the electric energy released during the discharge. If the breakdown of adenosine triphosphate, as suggested in these experiments, is the primary energy source in recovery, adenosine triphosphate should yield the energy for acetylation of choline. In agreement with this assumption, a new enzyme, choline acetylase, can be extracted from brain, which in cell-free solution under strictly anaerobic conditions, forms acetylcholine in the presence of adenosine triphosphate.[55-58]

"A third line of investigation in which the interdependence of

cholinesterase activity and conduction can be demonstrated is based on the effect of cholinesterase inhibitors. If the breakdown of the membrane during the passage of the impulse is connected with the release of acetylcholine and the restoration of the resting condition requires the immediate removal of the active ester, then inhibition of cholinesterase should abolish conduction. It can be shown on a great variety of nerves, that eserine, a strong inhibitor of cholinesterase, abolishes the action potential. The enzyme-inhibitor complex in this case is easily reversible. In agreement with the reversibility of the chemical process, the nerve and muscle action potentials reappear when the eserine is washed out.

"Recently a new potent inhibitor of cholinesterase became known, namely, di-isopropyl fluorophosphate (DFP). This compound, in contrast to eserine, destroys cholinesterase irreversibly. The rate of irreversible destruction depends, however, on several factors like temperature, concentration, etc. A most striking parallelism can be demonstrated between the rate of destruction of cholinesterase and the rate of abolition of the action potential. The close correlation between the two events can be established as a function of time as well as of temperature. The rate of irreversible inactivation of the enzyme *in vitro* at a given temperature coincides with the rate of irreversible inactivation of the enzyme and the irreversible abolition of the action potential in nerves. The experiments have established conclusively that the activity of the enzyme is inseparably associated with the conduction in nerve and muscle.[59, 60, 61]

"Prostigmine, another cholinesterase inhibitor, which is *in vitro* as strong as eserine, has no effect on nerve and muscle conduction. It can be shown on the giant axon of Squid that prostigmine, in contrast to eserine and DFP, does not penetrate the lipoid membrane surrounding all nerves (whether myelinated or unmyelinated).[62] Prostigmine is, like acetylcholine and curare, a methylated quaternary ammonium salt. Such compounds are not soluble in lipoids. This may explain why these compounds act only on the nerve endings where no lipoid membrane is found, but do not affect muscle or nerve fiber. The peculiar ability of the synapse to react to certain compounds, as first demonstrated for curare by Claude Bernard, was the basis for the assumption of a special mechanism for the propagation of the impulse across the synapse. In the light of recent biochemical and biophysical findings, this peculiar ability may be attributed to a *difference in the anatomical structure* rather than the underlying basic mechanism. Recent observations of Arvanitaki, Bullock, Eccles and others, support the assumption that the flow of current is the propagating agent across synapses, as it is along the fibers. In the pre- and post-synaptic

membrane, however, acetylcholine will most likely have the same role as in the active surface membrane of the fibers."

The precise mechanism whereby the flow of current acts in the effector organs is still uncertain. It might produce chemical change locally, or transient differences in the charge or the orientation of colloidal particles or macromolecules. As Volta long ago showed, the contraction of a muscle follows its electrical stimulation; but muscular relaxation follows cessation of the nerve impulses: merely to stand upright requires the subconscious innervation of many muscles. When unconsciousness supervenes (as in narcosis or fainting), the nerve impulses cease, the muscles relax, and the person falls limp. In the "rest cure" for tuberculosis, where it is desirable to keep exercise at the lowest possible level, the patient reclines in a nearly prone condition. Actually, the nerve impulses reach muscles at the rate of about 100 per second, so that the contraction of the muscles involves great numbers of "twitches." When age or disease alters the tempo of the twitches, tremor may become evident. Temporary nervous shock often causes trembling.

Professor George H. Parker of Harvard University has investigated the activity of various chemical activators, originally termed *neurohumors* by Henri Fredericq, which "really act as hormones over shorter or longer ranges." In discussing the nervous control of color in fishes, Parker goes back to Pouchet (1876), who showed that if the integumentary nerves of a turbot are cut, the melanin granules in its melanophores undergo a *dispersion,* spreading out the color and darkening the skin. Parker further observes that there are two classes of neurohumors: *hydrohumors,* soluble in water and therefore found in blood, lymph, etc., and *lipohumors,* soluble in lipoids, fats, oils, fat solvents, etc. He finds[63] that the color changes of catfishes are controlled by three chief neurohumors: intermedin from the pituitary gland; acetylcholine; and a concentrating neurohumor (probably adrenaline) from nerve fibers controlling, respectively, concentration and dispersion. Acetylcholine induces dispersion of melanophore pigment; adrenaline causes the reverse. Both are lipoid-soluble, and acetylcholine, in its fatty retreat, may thereby be protected from destruction by cholinesterase; in fact, it accumulates to such an extent that its effects may persist after nerve action has ceased, and it may be extracted in measurable amounts from dark fish skins. "Thus the fatty or lipoid substances in the animal body may serve as storage

reservoirs for agents that may be of first consequence in the animal economy."

Francis B. Sumner[64] has discussed the quantitative changes in pigmentation resulting from visual stimuli in fishes and amphibia. We thus get an idea of how and why flat-fishes, chameleons, etc., change color in response to their surroundings. This is related to the seasonal color changes in the hair of some animals (*e.g.*, ermines, hares, rabbits), brought about as Bisonette and others have shown, by seasonal variations in the amount of sunlight, acting through variations in the amounts of pituitary hormones secreted. If the animals are kept in illuminated cages, they develop summer coats even in winter.

Since the chemical units represent mainly consequences of catalytic activity, it would appear that the differences described probably stem back to the original cellular biocatalysts.

In quite another field, we find remarkable evidence of chemical control in bees. In full season a queen bee may lay in excess of 2,000 eggs daily, more than her own weight. Unfertilized eggs give rise only to drones (males), but the fertilized eggs may give rise to workers (sexually immature females) or to queens: nutritional difference decides the outcome. Townsend and Lucas state:[65]

"All female larvae are fed on royal jelly for the first 2-3 days after hatching and during this period their anatomical development is similar. Only the queen continues to receive this special diet. Any larva from a fertile egg, if given royal jelly throughout its larval period, develops sexually so that it becomes a perfect or true female bee, or what is called a queen; otherwise, the larva develops into a sexually immature worker. The queen is structurally much the same as the workers but with these important differences: the pollen-gathering apparatus remains undeveloped, the mouth parts and sting are modified, while the spermotheca and ovaries are highly developed."

These authors separated royal jelly into four fractions and found some evidence that the physiologically active material responsible for the sexual development of the queen bee is in the ether-soluble fraction. More recently Pearson and Burgin[66] report that bee royal jelly is the richest known source of pantothenic acid, its 35.8 per cent dry substance containing from 378 to 618 (average, 511) mg of pantothenic acid per gram.

In discussing ants, G. H. Carpenter stated:[67]

"One of the most interesting features of ant-societies is the dimorphism or polymorphism that may often be seen among the workers, the same species being represented by two or more forms.

Thus the British 'wood-ant' (*Formica rufa*) has a smaller and a larger race of workers ('minor' and 'major' forms), while in *Ponera* we find a blind race of workers and another race provided with eyes, and in *Atta, Ecitron* and other genera, four or five forms of workers are produced, the largest of which, with huge heads and elongate trenchant mandibles, are known as the 'soldier' caste. The development of such diversely formed insects as the offspring of the unmodified females which show none of their peculiarities, raises many points of difficulty for students of heredity. It is thought that the differences are, in part at least, due to differences in the nature of the food supplied to larvae, which are apparently all alike. But the ovaries of worker ants are in some cases sufficiently developed for the production of eggs, which may give rise parthenogenetically to male, queen or worker offspring."

Discussing the role of chemoreception in the ability of rattlesnakes to recognize ophidian enemies, C. M. Bogert[68] states that although rattlesnakes when confronted by man or by domesticated animals, normally assume a coiled defense attitude, with head raised ready to strike, immediately after exposure to ophiphagous king-snakes they react to contact and to visual stimuli only by assuming a characteristic "king-snake defense posture": head and tail flat on the ground, with the body arched to a high loop with which to strike a defensive blow. Rattlesnakes assumed this characteristic posture when placed in a receptable that had previously held king-snakes, and also when a clean stick that had been rubbed on the back of a king-snake was held near. The activating substance is apparently taken up by the tongue and conveyed to Jacobson's organ, which contains olfactory cells; and the response was evoked by visual or by contact stimuli as long as three hours after receipt of the original olfactory stimulus. Apparently some trace of a volatile substance powerfully conditions the behavior of the rattlesnake.

H. S. Raper and collaborators have shown[69] that the enzyme tyrosinase catalyzes the conversion of tyrosine (an amino acid constituent of some proteins) into 3, 4-dihydroxyphenylalanine, called *dopa* for short. Dopa is then oxidized by the same enzyme to a red indole derivative which spontaneously changes to *melanin*, a pigment which gives hair and skin a dark brown or black color. Factors affecting the activation of the precursor of tyrosinase have been studied by J. H. Bodine and collaborators.[70] The proenzyme separates in the aqueous layer obtained by ultracentrifuging mashed grasshopper eggs, and is activated when added to

the oily or lipid layer. Since chloroform, urethane, urea, heat and detergents (Duponol, Areosol) all produce the same final effect (active tyrosinase), Bodine states:

"The enzyme, tyrosinase, is protein in nature and as such shows all the characteristic properties of this chemical group of compounds. It, therefore, would seem logical to expect that activation may possibly be related to or dependent upon some physiochemical properties of this protein molecule and that the various activators employed bring about just such changes. Without adding unnecessary details in the way of experimental data, it may be stated that the activation of the melanin-producing enzyme in the present studies is thought to be brought about by the selective adsorption and orientation of the pro-enzyme molecules by the activating agents." Activation ceases to increase when the activator surface is occupied, either by pro-enzyme, or by another adsorbed protein.

The black spots on a Dalmatian dog appear where the local cells enable the melanin-producing process to occur, the remaining hairs being white. L. Earle Arnow[71] found that in the presence of oxygen, tyrosine may be converted by ultraviolet light into dopa, which is then changed into melanin by an oxidase. Sun-tan appears to be so caused in some persons. A curious case of inhibition of skin pigment formation appeared in a large tannery, where a considerable number of workers (about 50 per cent, many of them Negroes) developed white patches on their skins. Investigation showed that the rubber in some new gloves used to protect the workers' hands from an acid solution, had contained the monobenzyl ether of hydroquinone as an antioxidant, and that this substance also inhibits melanin formation; so that when the melanin originally present in the skin is absorbed or otherwised removed, the skin turns white (leukoderma). When the cause was removed the skin slowly returned to its normal shade, indicating that the pigment-forming mechanism was merely inhibited by the antioxidant, not destroyed. Normal skin color thus seems to represent a balance between pigment formation and removal.

E. L. Tatum and G. W. Beadle state:[72] "The development of eye color in *Drosophila* is known to be controlled by specific diffusible substances designated as v^+ and cn^+ hormones." By growing certain bacteria on an agar medium containing dead yeast, sugar, and *l*-tryptophane, "this bacterially produced v^+ hormone has now been obtained in a pure crystalline state," with "an activity of approximately 20,000,000 v units per gram when a solution is injected into vermilion brown test larvae."

An indication of how chemical control by trace substances may dominate fertility and thereby contribute an important factor to the course of evolution, is found in the Golden Rose strain of Petunia, which is completely self-sterile under natural conditions. Microscopic examination shows that its pollen tubes grow slowly, and that even before the most rapidly growing tubes reach half way down to the ovary, an abscission layer forms and blocks the way. Yasuda found that the placenta in the ovary of *Petunia violacea* secrets a "special substance" which diffuses into the style and completely inhibits pollen germination and tube formation. W. H. Eyster[73] found evidence that the ovarian secretion of the Golden Rose Petunia "which renders the plant self-sterile, can be transferred to other plants and renders them cross-sterile with pollen from self-sterile plants." Golden Rose Petunia can be self-fertilized by two methods: (1) "If flower buds which are beginning to develop anthocyanin in the petals are opened and pollenated with pollen from fully opened flowers from the same plant, seed capsules containing viable seeds are produced"; similar results were found by Yasuda[74] who calls this homo-pollination; (2) by spraying the flowering plants with a solution of ten parts of α-naphthalene acetamide in one million parts of water.

"Flowers which are sprayed with this solution immediately before or shortly after they have been self-pollinated produce seed capsules filled with viable seeds in exactly the same way that normal self-fertile plants of other strains produce seeds. Obviously α-naphthalene acetamide neutralizes the effect of the ovarian secretion which diffuses into the style and inhibits or greatly retards the growth of the pollen tubes."

Dwarfism in certain plants (*e.g.*, maize, pea) has been shown to be due either to deficiency of auxin (growth hormone) or to its destruction by oxidative enzymes. J. van Overbeek[75] further found that "laziness" in maize (a prostrate habit of growth) is consequent upon higher concentration of auxin in the upper part of the plant, whereas normally the reverse is true. He concludes: "It is thus evident that the lazy gene interferes with the auxin distribution in the stems which normally takes place under the influence of gravity."

While it has for some time been known that over about 1.5 parts per million of fluorine in drinking water generally produces a discoloration of the teeth known as "mottled enamel" (endemic dental fluorosis), Dr. H. Trendley Dean and collaborators[76] have found that fluoride levels of less than 1.0 ppm were accompanied by a corresponding increase in dental caries. While the function of fluorine has not yet been established, it seems possible that the liberation of fluorine ions locally, by acid-producing bacteria would result in the inhibition or death of the bacteria if the fluorine concentration became sufficient

before material attacks on the tooth had resulted. The fluorine probably also gives the tooth a denser and less readily attacked structure. "Bone gives an x-ray diffraction pattern similar to that of the mineral apatite, the unit structure of which contains $Ca_{10}(PO_4)_6F_2$. Various substitutions, such as $(OH)^-$ for F^- and Mg^{+2} for Ca^{+2}, are known to occur in the apatite lattice without producing significant changes in the diffraction pattern."[77]

Wide use is made of ethylene gas (about 1 part per 1000 of air) to "color up" fruits and vegetables which must be shipped more or less "green" in order to stand handling and avoid spoilage. Bananas, melons, pineapples, persimmons, tomatoes, oranges, apples, pears, etc., are so treated, the natural coloring processes being greatly accelerated. Celery may be thus blanched, and the hulls loosened from "stick-tight" walnuts. The diverse changes seem to be dependent upon initiating or facilitating specific enzymic changes.

Spraying apple trees with soluble thiocyanates, though it causes "spray burn" and a chlorotic condition of the leaves, tends to increase the red color of the fruit (blush) and to turn the green ground color toward yellow, both features desirable to consumers.[78] The red color is due to idaein, a glucoside which, on hydrolysis, yields cyanidin and galactose. There is some evidence that spraying apples with naphthaleneacetic acid and related compounds tends, with some varieties, to reduce the percentage of windfalls, apparently by firming the stems.[79]

D. W. Wooley has shown[80] that the mouse requires a new "vitamin" for normal growth and maintenance of hair. The facts indicate "that the mouse anti-alopecia factor is inositol or its derivatives. They suggest that inositol exists in liver in alkali-labile combination with a large molecule which renders the former non-dialyzable."

These scattering instances, which might be multiplied many times, illustrate how devious, various, and potent may be the effects of small amounts of substances, which, until comparatively recent times, were generally considered "negligible" in reports of chemical analyses. Their effects are often expressed through catalysts.

Biocatalyst Systems or Chains

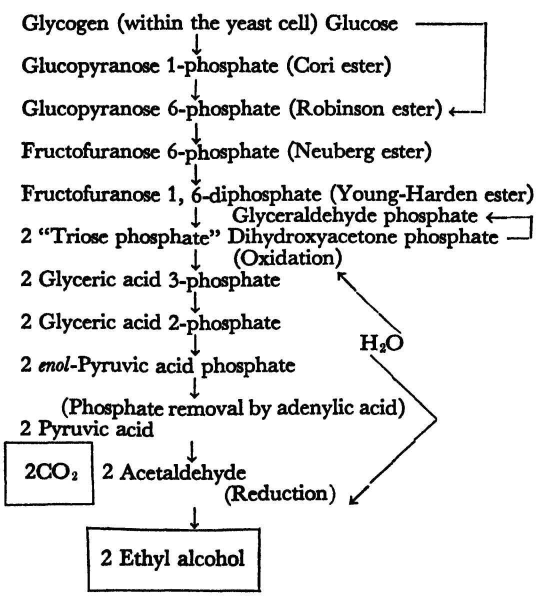
It is we who are simple, not nature. The physico-chemical happenings at various structural levels, which underlie the phenomena we observe, are numerous, intricate, often obscure, sometimes unsuspected. The complications involved in the structure, lability, and functioning of individual enzymes are magnified in cells, tissues, and organisms, where groups of catalysts and coöperative chemicals form chains or systems which have not yet been

constructed for experiments *in vitro.* Isolated enzymes, supposed to be pure, have sometimes proved to be mixtures, and it is always a question as to how far experiments with pure enzymes may be applied to an intact biological unit. Liver slices energetically oxidize certain of the lower fatty acids, but when the liver is minced this power immediately disappears.

As an illustrative instance, we may take the fermentation of glucose by yeast,[81] widely practiced and long studied. Present experimental data justify the belief that underlying the extremely naive equation

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_5OH$$

the following catalyzed system is operative (end products shown in rectangles):

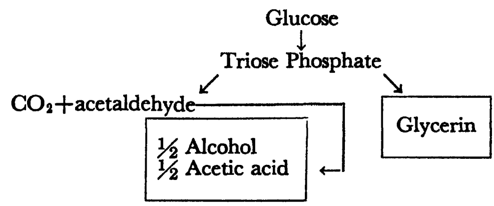


The successive steps tabulated are catalyzed by specific enzymes, and many of the changes have been shown to be reversible. Besides the final products (ethyl alcohol and carbon dioxide), a small amount of glycerin is also formed. Since the formation of Cori ester from glycogen is inhibited by glucose, enzymic formation of glycogen within the yeast cell may serve to restrict glucose concentration as glucose diffuses into the yeast cell.

If sodium sulfite is added to a yeast fermentation, the formation of alcohol practically ceases and the small percentage of glycerin which occurs in normal fermentation increases so that it becomes a main product, with acetaldehyde and carbon dioxide. The

acetaldehyde is fixed by the sulfite, the CO_2 is liberated, and the glycerin remains; during World War I Germany produced glycerin by fermentation processes. Bell[82] states that the traces of glycerin in normal fermentation are due to the "mutase" system in the yeast cells. It seems that, normally, acetaldehyde acts as a hydrogen acceptor in the anaerobic oxidation of triose phosphate; but when the acetaldehyde is removed by the sulfite present, another molecule of triose phosphate can act instead. Since the former reaction is the more rapid, little glycerin is formed when acetaldehyde is present.

By carrying out the fermentation at an alkaline pH, Neuberg found that the breakdown of the glucose yielded glycerin, alcohol, and acetic acid, according to the following scheme, which is not yet understood in detail:



Not only does the apparently simple yeast cell contain a considerable number of specific enzymic catalysts and collaborating molecules, but it can be "trained" to ferment galactose,[83] by adding small and continually increasing percentages of this sugar to the fermentation mixture. Evidently yeast can "manufacture" new enzymes to meet new situations; probably the new substances add their own specificity to a system of molecules to make a new prosthetic group.

Modification of Biocatalysts

The writer has taken the view that biocatalysts are subject to *modification,* which involves the fixation, at an active catalyst area of a gene or other catalyst unit, of some particle (electron, ion, atom, molecule, or colloidal particle) which changes the nature and/or the rate of the catalytic change occurring there. A *limiting case* under this general principle would be the *formation of a new catalyst area* by the fixation, *e.g.*, at a protein surface, of a prosthetic group. In the case of genes a change in catalytic output may arise from an intraparticulate change in the gene itself (known as a *point mutation*), or because of some chromosomal

upset (*e.g.*, inversion, translocation, crossing over) which places the gene in different surroundings (*position effect*). If heritable and non-lethal, both these types of change generally lead to new mutant forms of plants or animals. The modification of enzymes within a cell, or the formation or introduction of new enzymes there, could lead to catalytic variations of chemical output which might simulate the effects producible by a point or a chromosomal mutation; but the result would be transient unless sufficient of the modifiers and prosthetic groups to maintain it were supplied or produced. Another possibility is that a specific area may serve as a template or mold against which may be formed specific catalytic molecular structures or plaques. Still another possibility is the local establishment of ionic or trace-substance conditions favorable to the formation of new enzymes, *e.g.*, by adsorptive fixation of a prosthetic group by a carrier. Viruses and bacteriophages reproduce themselves in cells, thus (where they exert catalytic action) behaving *as if* they were free-living genes. What is said of genes may be applied with equal force to mitochondria; for whether these are symbionts or cytoplasmic inclusions, they apparently reproduce and are efficient in directing chemical changes within the cell (either directly or through enzyme formation), and behave somewhat as if they are free-living genes or gene groups.

The stability of genes and the efficiency of the protections surrounding them are evidenced by the regular and orderly sequences normal to life, which shows that gene mutations are relatively quite rare, and abnormal gene or catalyst modifications unusual. Since non-lethal gene mutations may be transmitted by heredity, they are basic factors in evolution; for beneficial mutations tend to survive and dominate, whereas harmful ones tend to die out. If mutation makes a gene (or other catalyst) more susceptible to an abnormal modification, that is one way in which the effect of a mutation may become evident.

Bacterial Dissociation and Transformation[84]

The colonies of most bacteria that have been studied may appear in a rough, corrugated form (R), or in a smooth, glistening, drop-like form (S). The change of a culture from R to S, or *vice versa*, is known as *dissociation*. The methods used to produce dissociation involve, mainly, change in the usual growth medium: (1) by the addition of sera, normal or immune; (2) by addition of

definite chemical agents (*e.g.*, $LiCl_2$, $FeCl_3$, sodium taurocholate); (3) by change in pH; (4) by animal passage, a method much used, involving considerable, though unknown, changes in the culture medium. For example, Alexander-Jackson[85] was able to change two strains of human tubercle bacilli of R form over into S by the addition of 0.0004 per cent of ferric chloride to Bordet-Gengou medium. As a rule, the S form is relatively more virulent than the R form of the same strain. At the laboratory of the New York City Board of Health the virulence of test strains of pneumococci is maintained by daily passage through mice, to give an example of the well-known effect of animal passage, which also exerts changes in the virulence of viruses; *e.g.*, the virulence of smallpox to man is reduced by passage through cattle.

On the other hand Herald R. Cox[86] found that the virulence of a *Dermacentor variabilis* strain of Rocky Mountain spotted fever was enhanced, as against guinea pigs, upon growth in the yolk sacs of developing chick embryos. The virulence decreased after about 50 passages, and after about 100 passages the strain, while causing slight or no reaction in the guinea pigs, made them "solidly immune to massive doses of highly virulent strains." See, also, R. E. Green[77] on the nature of virus adaptations.

Griffith,[87] working with white mice, discovered the interesting fact that S forms of pneumococci can be transformed from one specific type into other specific types through the intermediate stage of the R form. Since the specificity of the types involves the formation of specific substances, (especially, as Heidelberger[88] has shown specific carbohydrates), it seems obvious that the catalysts of the pneumococci in Griffith's experiments must have undergone a change; either old catalysts were transformed, or new ones were formed.

Dawson and Sia,[89] working *in vitro*, found that R forms of pneumococci derived from S forms of one specific type (Type II), can be transformed into the S forms of other specific types (Types I or III), by growth of small inocula of the R form of Type II in media containing vaccines prepared from heterologous S cultures. It seems possible that in this case the R form contains an enzymic *carrier* which has been deprived of its prosthetic group, but which takes up *a different prosthetic group* from the new vaccine-containing medium. That these R forms are more labile is brought out by Sia and Dawson,[90] who state: "R cultures possessing only slight degree of R stability are most suitable for transformation purposes by *in vitro* procedures."

Drs. Oswald T. Avery, Maclyn McCarty and Colin MacLeod, of the Rockefeller Inst. for Medical Research, found that the substance induc-

ing the transformation of pneumococcal types isolated from Pneumococcus Type III, is "in the form of a desoxyribonucleic acid fraction. The data obtained by chemical, enzymatic, and serological analysis, as well as by electrophoresis and ultracentrifugation of the purified material, strongly suggest that the nucleic acid is itself responsible for the biological activity." Minute amounts of this substance can cause uncapsulated "rough" variants of Pneumococcus Type II to acquire the capsular structure and type specificity of Pneumococcus Type III. Ascorbic acid (and other autoxidable compounds, *e.g.*, catechol, hydroquinone, *p*-phenylenediamine) inactivate the transforming substance.

With ascorbic acid inactivation is catalyzed by traces of cupric ion; this is prevented by the presence of sulfhydryl compounds, which can restore activity under certain conditions. Activity is irreversibly destroyed by highly purified preparations of the specific enzyme desoxyribonuclease, capable of depolymerizing authentic samples of desoxyribosenucleic acid of animal origin. The enzyme requires the presence of a metallic activator (in nature magnesium, though manganese in like molar concentration is equally effective). The specific transforming substance isolated from pneumococci of types II and VI, in addition to type III, consists of desoxyribonucleic acid as the biologically active substance. "Although the individual desoxyribonucleates of different types cannot yet be distinguished from one another on chemical grounds, the selective specificity they exhibit in inducing transformation is difficult to interpret save in terms of individual differences in chemical structure and molecular configuration."[91]

As pointed out in Chapter 3, note 1, naturally occurring carotenoids having seven double bonds may have 128 possible stereoisomers. With 12 stereochemically effective double bonds the number of possible isomers rises to 2800 for symmetrical chains, and 4096 for unsymmetrical chains. How stereoisomerism can affect the properties of substances having the same ultimate chemical analysis may be seen from the following excerpts from the review of L. Zechmeister of the California Institute of Technology.[92]

"A long conjugated double-bond system offers many spatial possibilities, but the synthetic as well as the natural polyenes are usually all-*trans* compounds. . . . This configuration can be easily altered by various thermal, photochemical, or catalytic treatments. Upon such treatment the stereochemical uniformity of the molecules disappears and a complicated mixture of *cis-trans* isomers is formed, in which ordinarily the unchanged portion of the initial

all-*trans* pigment still predominates. Theoretically, each possible stereoisomer must be present in such an equilibrium mixture if only in minute quantity.

"The chemical characteristics of the stereoisomers of a given carotenoid are very similar, but fortunately the adsorption affinities are highly dependent on configuration, perhaps more so than any other known physical property. Therefore, the Tswett method of chromatographic adsorption is the only effective technique for the separation and study of the stereoisomers.[93]"

It appears that many biologically important compounds may have superimposed upon their basic chemical structure physical or physicochemical impressions or distortions which can affect their specificities in the catalytic field of activity. While every substance has a chemical structure, this does not preclude the simultaneous formation of a superstructure in the case of complicated molecules or molecular groups.

In cultures of *Hemophilus influenzae, Escherichia coli,* and *Streptobacillus moniliformis,* the germination of "large bodies" has been observed, and the descendants of these "L type" colonies, instead of resembling the parent organism, are similar in both morphology and development to the pleuropneumonia group of organisms. Such "L type" colonies come from strains which are rare in most species, but they were also observed in cultures of a *Flavobacterium,* of *Bacteroides funduliformis,* and of the gonococcus, and have been isolated in pure cultures from the two former. L. Dienes[94] believes that they represent a variant type, in which case the variability of bacteria extends much further than is commonly supposed. E. Kleinberger (references in Dienes' paper) thought that they represented symbionts, a view that Dienes opposes. The question is not finally decided.

Speaking of the enzymatic synthesis of polysaccharides, M. Stacey[95] states: "There is good evidence for the theory, here advanced for the first time, that the synthesizing enzyme remains *in combination with the polysaccharide* it has synthesized, and there is progressively built up a complex macromolecule in which comparatively short polyglucose chains are 'cemented' together by a nucleoprotein. In the case of one dextran from *Betabacterium vermiforme* (Ward-Meyer), the aggregating process can go on until the macromolecule is so large that it settles out of the solution in the form of granules. The separation of granular synthetic starch takes place in precisely the same manner and the product contains significant amounts of a nitrogenous constituent which could only come from the phosphorylase preparation."

Evidence that bacterial catalysts direct the formation of specific

polysaccharides appears in the statement of E. J. Hehre[96] and J. Y. Sugg that a serologically reactive polysaccharide of dextran nature was produced from sucrose by sterile filtered extracts derived from sucrose broth cultures of *Leuconostoc mesenteroides,* a Gram-positive coccus widely distributed on plants. "Rigorous controls were included to prove that this reaction occurred in the absence of microörganisms." The polysaccharide was recognized chemically and also by its ability to react with the anti-serums of types 2 and 20 pneumococci, as well as with the anti-serum of the homologous bacteria.

These and many other facts support the view that the specificity of all species, both plant and animal, stem from, or least involve, differences in the basic biocatalysts. The visible forms with which taxonomy deals, and even the specific substances which biochemists isolate and identify, are mainly an aftermath. The basis of species specificity and even of evolution rests largely on biocatalysts and their changes.

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[3] "Further information on this subject may be had in the article by Berzelius in Schumacher's Jahrbuch for 1836, pp. 88-97."

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[7] Presented before the Genetics Section of the American Society of Zoologists and the Botanical Society of America on Dec. 28th, 1928 (Science (1929), **70**, 508-510).

[8] "Catalysis and Its Industrial Applications," in "Colloid Chemistry," Vol. VI, Reinhold Pub. Corp., 1946.

[9] Berkman, Morrell and Egloff ("Catalysis—Inorganic and Organic," Reinhold Pub. Corp., 1940) give an extensive list of catalysts for many different types of chemical reactions, including hydrogenation and dehydrogenation, oxidation, hydration and dehydration, cyclization, halogenation and dehalogenation, hydrocarbon cracking, polymerization, alkylation, and isomerization.

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[15] Roughly, the following represent normal rectal temperatures:

36-38° C (96-101° F.)—man, monkey, mule, horse, elephant, rat, mouse.

38-40° C (100-103° F.)—cattle, sheep, goat, dog, cat, rabbit, pig.
40-41° C (104-106° F.)—duck, goose, turkey, owl, pelican, vulture.
42-43° C (107-109° F.)—fowl, pigeon, quail, sparrow, starling, bluejay.

The duckbill (*Ornithorhynchus paradoxus*, a monotreme), and the Australian anteater (*Myrmecobius faciatus*, a marsupial) represent forms intermediate between the cold- and the warm-blooded animals, their body temperatures averaging only 25° C (77° F.), and changing by about 10° C with an environmental change of 30° C. While the terrible consequences to the closely packed prisoners in the Black Hole in Calcutta were originally ascribed to lack of oxygen, it seems likely that lack of cooling in that stagnant atmosphere was a big factor. In hot weather we seek breeze or electric fans to help us cool off by transpiration. On the other hand, sheep and bees huddle together to keep warm, and I have seen "ladybugs" (actually, beetles) gathered together in masses for hibernation. For details and references, see Brody, "Bioenergetics and Growth," Chapter 11.

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[23] "Bioenergetics and Growth," p. 111.

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[25] $C+O_2=CO_2+94$ Cal*
$H_2-\frac{1}{2}O_2=H_2O+68$ Cal
$S+1\frac{1}{2}O_2+H_2O=H_2SO_4+142$ Cal
$NH_3+1\frac{1}{2}O_2=HNO_2+H_2O+79$ Cal (*Nitromosas*, nitrite producer)
$HNO_2+\frac{1}{2}O_2=HNO_3+21$ Cal (*Nitrobacter*, nitrate producer)

* The large Calorie (Cal or Kcal) is the amount of heat necessary to raise the temperature of one kilogram of water 1° C. The small calorie (cal) is one thousandth of a Cal.

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[29] To illustrate the importance of the carrier, it may be mentioned that Adkins, Richards and Davis found marked carrier effects in the catalytic dehydrogenation of hydromatic compounds containing a completely or partially saturated benzene, naphthalene or phenanthracene nucleus. In the case of decahydronaphthalene, the yields of naphthalene varied not only with the catalyst but also with the carrier.

Catalyst	% yield of naphthalene from decahydronaphthalene
Pt. on charcoal	87
Ni on charcoal	34
Ni on chromium oxide	78
Ni on kieselguhr	62
Ni on Alumina I	0
Ni on Alumina II	11
Ni on Alumina III	0
Ni on Alumina IV	36

Note: The charcoal was purified Norit. Alumina I was prepared through sodium aluminate; alumina II, III, and IV, by hydrolyzing alumina isopropoxide by three different procedures.

The authors remark that nickel on alumina and on kieselguhr are better catalysts for the dehydrogenation of substituted cyclohexanols than is platinum, because they show less tendency to induce condensations. Nickel on chromium was the most active catalyst in the case of resistant saturated hydrocarbons. In all, 28 representative compounds, including hydrocarbon alcohols, ketones, and ethers, were heated at 300° to 350° in the liquid phase under nitrogen, in the presence of the catalyst and also of benzene, which serves as a hydrogen acceptor or oxidizing agent. (J.A.C.S., 1941).

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Chapter 7

Immunology and Self-Saving Catalysts

Immunity itself must have begun with the development of the mechanism whereby organisms respond to the presence of infectious organisms by forming antibodies. But the science of immunology, which studies the extent, nature and mechanism of immunity, is of relatively recent development, and has been given extensive study by specialized groups of physicians, bacteriologists and chemists. As early as the 7th century A.D. person-to-person inoculation was used in China to protect against smallpox—a practice also known in Arabia. A full century before Jenner introduced vaccination in England, the Chinese were using cowfleas, an insect carrier, to give a protective attack of cowpox.[1] As a practical method, immunology dates from Louis Pasteur, who developed methods of protection against the dreaded anthrax and rabies by the use of weakened or attenuated infective agents. I reproduce here the signature of

As a small boy, he was the first person saved by Pasteur from rabies. This signature was obtained in 1926 at Institut Pasteur in Paris, where Meister was concierge, a living monument to the great chemist-bacteriologist-immunologist.

Since the precise chemical nature of the colloidal substances involved in immunological reactions was unknown, they were given names corresponding to their observable effects. The special nomenclature which developed is here outlined.

Substances are termed *antigens* if they can stimulate or cause a plant or animal to produce other substances, termed *antibodies,* which are able to react specifically with the inciting antigen. Time is required for the formation of the antibody, or *immunization,* as it is called; and an organism may inherit immunity to many antigens or may develop immunity to them, *e.g.,* either by

their proper injection or by their introduction through invading organisms or viruses in an attack of a disease. The U. S. Army uses immunization to protect against cholera, smallpox, tetanus, typhoid fever, typhus, and yellow fever, and sometimes against diphtheria, influenza and some forms of pneumonia.

Chemically, antibodies are proteins present in blood serum, whose physical properties class them primarily with the globulin fraction of the serum; they are globulins according to electrophoretic studies by Arne Tiselius and E. A. Kabat;[2] but their peculiar specificities are not necessarily and exclusively due to chemical differences, as so many assumed. The paper and metal industries show how many lasting contours may be impressed on sheets without involving chemical change. The ultracentrifugal work of The Svedberg[3] (Nobel prize, 1927) indicates the molecular weights of some antibodies and other large molecules:

Serum globulin (horse)	68,000— 167,000
Serum albumin (horse)	70,000
Antipneumonococous globulin (horse)	910,000
Serum globulin (man)	176,000
Antipneumococcus (man)	195,000
Lactoglobulin	41,000
Pepsin	35,500
Insulin	46,000
Catalase	250,000
Urease	480,000
Hemocyanins (various sources)	400,000—8,700,000

But these "molecules" are not spheres. A. Neurath[4] makes the following estimates: Rabbit pneumococcus antibody, 37Å x 338Å; horse pneumococcus antibody, 20-47Å x 950Å. J. R. Marrack reports[5] that on immunization there may be an increase in serum protein, mainly in the globulin fraction.

Apart from these *immune bodies* developed from the introduction of the specific antigen, the blood serum may carry, as the result of genic or humoral inheritance, a number of *natural antibodies* to some antigens; and such inherited immunities may in some cases be important factors in survival. Huge numbers of various American, Mexican, and South American tribes succumbed to smallpox, and measles decimated the natives of Pacific islands among whom natural immunity to this disease was rare if not non-existent.

The envelopes of erythrocytes have areas of what appear to be specific polysaccharide substances which act as agglutinogens and can react with corresponding agglutinins. K. Landsteiner's pioneer work (1900-1) has been extended, and there are now recognized, in humans, the following types: A, B, AB, O; subgroups of A and AB; the M. N, and P agglutinogens of K. Landsteiner and P. Levine[6]; and the Rh factor of K. Landsteiner and A. S. Wiener.[7] These agglutinogens can be distinguished by tests with suitable antisera prepared by immunizing human beings or animals. Agglutination generally occurs, but in some cases hemolysis of the red blood cells takes place. A. S. Wiener states: "In place of the original, single Rh factor, transmitted by a pair of allelic genes, *Rh* and *rh*, three Rh factors are known at the present time, together with a so-called Hr factor, and these, in combination, give rise to a large series of different varieties of Rh agglutinogens, determined by a corresponding set of at least ten or more allelic genes."[8] In fact, all the different "blood groups" seem to be distributed genetically, and the bloods of animals close together on the phylogenetic scale, (for example, man and chimpanzee, rat and mouse) are immunologically related. If a goat is immunized by injections of red blood cells of man, rabbit, and pigeon, the blood cells of each of these three animals will absorb from the goat's serum only its own specific agglutinin.

The immunological consequences arising from differences in blood groups have much to do with incompatibility of blood used in making transfusions, so that a suitable blood donor must be selected. In plasma transfusions, blood group differences can usually be disregarded. Transplantations of tissues may also fail because of such or similar incompatabilities. In some cases this may cause the death of a child *in utero*, or shortly after birth (*Erythryblastosis fetalis*), unless the blood of the infant which has been damaged by the maternal antibodies, can be replaced soon enough by compatible blood.

Embryonic and new-born animals usually have *passive immunity* to certain antigens, acquired by diffusion of parental antibodies through the placenta, or else by their deposition in the egg. Embryos and young are able to form antibodies very slightly, if at all; and before the new-born animal develops this power and thus establishes its own *active immunity*, its passive immunity begins to drop off. Hence the very young are generally susceptible to many antigens, especially to the viruses and bacteria of "children's diseases."[9] Passive immunity is now commonly given children by injections of antibodies, of antigen-antibodies,

or of modified antigens (*e.g.*, diphtheria toxoid). Vaccination (from *vacca*, a cow) infects one with the usually very mild cowpox, and thereby establishes an active immunity against smallpox.

While most antigens are proteins with large colloidal molecules, certain carbohydrate and lipo-carbohydrate substances have been recently shown to act as antigens. Simpler proteins do not serve as antigens, for example, protamines consisting mainly of complexes of diamino acids, and gelatin lacking tryptophane which contains a benzene ring.

Toxins are *poisonous antigens.* Many antigens, such as egg albumin, casein, and animal sera, are not *toxic* in the small quantities needed for immunization. As Prof. J. J. Abel pointed out, the Greek word *toxikon* originally meant "of or belonging to the bow," a meaning that still survives in the word *toxophilite,* a lover of the bow or archery. The word was carried over to the arrows shot from the bow, and then to the poison into which the arrow heads were usually dipped. Many substances like phenol, arsenic, prussic acid and morphine which do not elicit the formation of antibodies, should be called poisons, not toxins.

Many toxins are formed by pathogenic microorganisms. *Endotoxins* come from the bacterial body itself on breakdown (typhoid bacillus), while *exotoxins* are substances formed by and excreted by the bacteria (*e.g.*, diphtheria). *Snake venoms* and the highly poisonous *toxalbumins,* such as abrin (from the jequirity bean) and ricin (from the castor bean) induce the formation of specific antibodies. Some toxins, when altered chemically or physically, become non-toxic but still preserve the power to form antibodies to the original toxin, for example, diphtheria toxin treated with formaldehyde and alum. A modified toxin of this sort is known as a *toxoid,* and its immunizing power may well be due to the slow liberation of the toxin or a closely similar substance.

Synthetic Antigens

This interesting field was developed by Dr. Karl Landsteiner (Nobel prize, 1930), who coupled organic substances of known chemical constitution to proteins by a process known as diazotization. For example, when metanilic acid (*m*-aminobenzene sulfonic acid) is treated with nitrous acid, the amino group yields a highly reactive diazo group, which chemically fastens the metanilic acid molecule residue to the protein. This is a counterpart of the

process long in commercial use for making certain dyes or colors; thus *para red* is made by diazotizing *p*-nitraniline and adding beta-naphthol.[10]

Landsteiner found that his newly synthesized protein addition compounds could evoke the formation of specific antibodies, but that the specificity depended largely upon the nature of the molecule attached, which he termed the *hapten.* While haptens, of themselves, are unable to cause antibody formation, when they are attached to the protein they determine to a large extent the specificity of the antibody formed.* The fact that haptens which are very similar chemically, interact serologically ("cross-reactions"), supports the view that the specificity of antibodies and of antigens is determined by their outwardly directed electronic fields, which naturally vary with change in chemical and physical structure.

Reactions Between Antigens and Antibodies

Most immunological reactions fall into one of the following groups:

(1) *Toxin-antitoxin neutralization:* If properly made, a mixture of toxin and its specific antitoxin is innocuous. A toxin formed in or introduced into an animal can be neutralized by its specific antibody, *if administered in time.* Thus diphtheria antitoxin must be given before the diphtheria toxin, produced by the invading bacteria, has been irreversibly fixed by certain nerve cells in the medulla. Death from paralysis of the heart and the respiratory system may follow injury to these cells, and even in cases that recover, temporary paralysis of the limbs may occur. Injections of toxoid or of toxin-antitoxin mixtures are now generally used to establish immunity to diphtheria. As far back as 1909, Dr. J. G. M. Bullowa and the writer followed in the ultramicroscope the mutual coagulation of diphtheria toxin by diphtheria antitoxin and of tetanus toxin by tetanus antitoxin. But neither toxin formed a coagulum with the antitoxin specific to the other one.[11]

(2) *Precipitin reactions:* When a suitable amount of a soluble antigen is mixed with the blood serum of an animal immunized by injections of this particular antigen, a precipitate appears, and generally is visible. This is known as the Ramon test.[12]

(3) *Agglutination reactions:* When an antigen having visible

* This recalls the action of prosthetic groups in enzymic catalysts.

particles (*e.g.*, bacteria, red blood cells) is mixed with a serum containing the specific antibody elicited by injections, the particulate antigen agglutinates, or forms flocs which usually settle out. Motile antigen cells, such as typhoid bacteria or sperms, lose their independent motility on coherence, though some cells in a floc may show some motion for a while. This is seen in the Widal test for typhoid fever, for in the serum of a person who has, or has recently recovered from the disease, live, motile typhoid bacilli agglutinate and soon dissolve—the phenomenon termed lysis (solution).

(4) *Lysis:* Antibodies that can dissolve or disintegrate a particulate antigen are termed *lysins.* Special names indicate the kind of antigen dissolved: bacteriolysis, for bacteria; cytolysis, for cells; hemolysis, for blood corpuscles.

Complement or Alexin

After 15 minutes' heating at 55° C an immune serum capable of causing lysis of cholera vibrios loses this power, but regains it on the addition of some unheated non-immune serum. Obviously, both immune and non-immune sera contain a factor essential to the action of the lysin. Prof. Jules Bordet of the University of Brussels (Nobel prize 1922) termed this factor *alexin* (from the Greek meaning *helper*), while Prof. Paul Ehrlich of the University of Berlin (Nobel prize 1908) called it *complement.* In the Wassermann test a normal serum furnishes the proper amount of complement, so that complete lysis occurs. With the serum of a person having syphilis, the complement is altered, bound, or "deviated" to a greater or less extent, so that lysis takes place only partially or not at all. Absence of lysis is recorded on an arbitrary scale as 4+.

Antibody Formation

Though antibodies are usually tested for in body fluids such as blood serum and spinal fluid, it is believed that they are formed within the cells. On bleeding an immunized animal, for example a horse, to extract diphtheria antitoxin, a new supply is continuously forthcoming. Since a small amount of antigen can produce an indefinitely large amount of specific antibody, it is obvious that the original antigen molecules cannot constitute the antibody, even in part. This led me to the view that the antigen formed a specific catalyst surface or template, against which a specific antibody of opposite contour could be molded. I have illustrated this notion in frequent private discussions and public lectures, by

pressing a sheet of tin foil against a coin and showing that the coin produces a reverse impression on the foil on the *near* surface. A *duplicate* of the coin contour is produced on the *off* surface, and the significance of this will be referred to presently. Free discussion for several years having disclosed no opposing evidence or reasonable alternative, a brief paper was offered for publication to several American scientific journals; but publication was refused on the advice of "referees." Realizing that the paper could not be published here because of this opposition, it was sent to *Protoplasma* and was published by this journal in October, 1931, under the title "Some Intracellular Aspects of Life and Disease." The following quotations are from this paper.

"Immunological specificity, like all other kinds of chemical specificity, is consequent on the outwardly directed electronic fields of the units involved in precipitation, agglutination, lysis, etc. The minimum sensitizing dose of egg albumin approximates 0.000,05 milligram; and in general the minute quantities of antigen demonstrable by immunological methods cannot be detected by any other known method. How shall we account for the potent effects of such incredibly minute quantities, and also for the fact that if an animal be bled, the temporary drop in the titer of antibodies in the remaining blood is retrieved or even surpassed? Furthermore, the blood of a non-sensitized animal may be used to replace the blood of a sensitized animal, without impairing the sensitivity of the animal or of its isolated tissues.

"These facts point to *the formation, within the cells themselves, of new specific catalysts which are able to direct the formation of antibodies.* Three possibilities present themselves as the method whereby specific antigens produce specific catalysts which in turn determine specific antibody formation: (1) modification of a gene; (2) modification of a non-genic catalyst; (3) fixation of the antigen particle by a non-catalyst cytoplasmic particle in such a manner that the combination functions as a specific catalyst. Nature may utilize any or all of these methods, and perhaps others unthought of at present.

"All three of these possibilities involve the idea that the antigen becomes *an essential part of the directive surface of a catalyst particle,* which would tend to determine the formation of particle groups *having essentially a reverse of the electrostatic charge pattern* of the active catalyst surface and therefore of the antigen, or else of particle groups which *can acquire essentially such a reverse*

pattern when they are detached and removed to some other part of the organism. Changes in hydrogen ion or other ion concentration might readily account for such detachment and changes, which would be in the nature of an electroversion. In its simplest form this concept may be illustrated by the following diagrams,* wherein positively charged areas are represented as depressions below, and negatively charged areas as elevations above, the dotted line representing neutrality. (See Figure 17).

"The applicability of Emil Fischer's well-known analogy of lock and key, which he applied to the fitting of enzyme to substrate, is at once manifest. That such an antibody particle would tend to unite with particles of its specific antigen seems obvious; and the neutral units would tend to flocculate if conditions would permit—presence of precipitating ions and absence of colloidal protectors. . . .

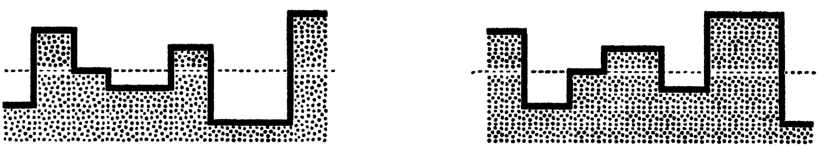


FIGURE 17. (*Left*) Active "antigen area" in modified catalyst. (*Right*) Oppositely changed area in antibody formed by modified catalyst. [Courtesy *Protoplasma*, Vol. 14, No. 2 (1931).]

"The effectiveness and lasting effect of minute quantities of antigens becomes comprehensible on the basis of this view, for in theory at least, one single molecule or colloidal particle would be sufficient to convert a cell or an extracellular catalyst into a potential producer of a specific antibody. Furthermore, there is no reason why large numbers of different antigens may not simultaneously or successively affect the same cell with its many thousands of genes and other catalyst particles—which corresponds with the experimental facts. As long as the catalyst-antigen complex continues to function to produce the specific antibody, so long will the production of immune bodies continue, despite bleeding. Variations in the duration of immunity would correspond to variations in the persistence of the antigen-catalyst complex while inability to establish immunity would indicate the non-formation of such a complex (or destruction of the antibody). All these phenomena appear in 'vaccinations,' a general term indicating introduction of antigens with the hope that immunity will result."

* The actual fields of force extend in three dimensions.

And in a more recent publication[12a] I wrote: "The functioning of the antigen 'mold' or 'template' may be crudely illustrated, at a much higher structural level, by pressing a piece of tinfoil against the surface of a coin.[13] The surface of the foil *next* to the coin acquires an impression which is the specific *opposite* or reverse intaglio of the coin pattern, while the *upper* surface of the foil acquires a surface pattern which *duplicates* the coin pattern. If something analogous occurs when a molecular plaque is formed against an antigen mold, the subsequent influence of the detached plaque would depend upon *which of the plaque surfaces remains exposed to the milieu,* if the plaque serves as adsorbent or is itself adsorbed. There could thus be formed a new surface like the mold, or a surface with a reverse contour, or modifications of either of these surfaces if the plaque is distorted on adsorption or is subject to enzymic or other biochemical attack.

"The separation of duplicated chromosomes during mitosis shows that forces exist which separate the duplicated gene-strings from each other. As N. K. Koltzoff[14] and C. B. Bridges[15] independently showed, the huge salivary gland chromosomes in the small grub that develops into *Drosophila,* which are enlarged or swollen to about 200 times normal size, appear to consist of a number, possibly 16 or more, of gene strings which, instead of separating, remain coherent and, when stained, show specific bands and structures at the loci of specific genes. On comparing the location of these bands with the gene maps developed from the data of geneticists, their matching indicates that we have before us what has been termed a 'genetic spectrum.'

"What forces normally separate each template gene from the new gene formed against it, so that the new chromosome may separate lengthwise from its originator? And what forces would determine the separation of our hypothetical plaque from the surface against which it was formed? While no simple or positive answer can be given, it must be recalled that small changes in ionic concentration (pH with protein units, C_{Ca} in the developing zygote) could be effective, as may also be the presence of small amounts of specific substances which act as "detergents," as Svedberg found with proteins and as Smith found with natural chlorophyll. Another possibility is suggested by the experiments of Goranson and Zisman[16] who found that when about 500 successive X-multilayers of calcium (or barium) stearate were deposited upon an ebonite 'probe,' the polymolecular layer spontaneously detaches itself.[17] Possibly the cohesive surface forces diminish as the layer becomes thicker, and are no longer effective when the deposit reaches a critical thickness. It is not unreasonable to envisage the possibility that specific natural proteins, carbohydrates, etc., may be thus formed at the surface of specific catalysts as templates, and float away to become effective units elsewhere. A

contrary effect seems to be produced by colchicine, which causes chromosome doubling or polyploidy, apparently by interfering with spindle formation during mitosis.

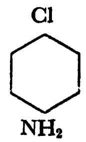
On the other hand C. C. Lindegren and C. B. Bridges[18] advanced the hypothesis that the surface of each chromosome (not necessarily the gene surface), may stimulate the protoplasm to form specific antibodies, which on being specifically adsorbed at the chromomere interface, renders it capable of adhering specifically to its partner chromomere in synapsis. Any two allelic chromomeres happening to touch "would be cemented together by the antibody junctions specific to themselves. The chromomeres which are on each side of the already agglutinated ones would then be more likely to touch and fuse, so that synapsis would proceed, zipper-like, from the first points of the homologous contact throughout the entire length of the chromosomes.

In this connection, it is interesting to consider the concept of "cohesive colloids." W. W. C. Topley, J. Wilson, and J. T. Duncan[19] found that when a heterogeneous mixture of bacteria is agglutinated by a heterogeneous mixture of specific antisera, each cluster of bacteria is homogeneous. Apparently each kind of bacterium becomes coated by a layer of its own specific antibody, and the bacteria are so specifically conditioned that each kind forms a lattice or clump of identical unions through the adsorbed antibodies. The submicroscopic particles containing glycogen isolated by fractional ultracentrifugation by A. Lazarow[20] from finely dispersed liver, appear to be aggregates of smaller glycogen particles held together by about 1 per cent of protein, a coacervating agent which "seems to parallel the action of insulin, because insulin is known to lower blood sugar and facilitate glycogen storage in the liver."

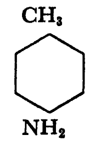
The Nature of Antigen-Antibody Reactions

"Out of the efforts to explain the diverse and often confusing phenomena appearing in immunological reactions, there arose an unfounded and totally unnecessary antagonism between those who attempted to explain all of them on the basis of simplified concepts of colloid chemistry and those who could see nothing in them but applications of the stoichiometric laws of classical chemistry, which had been developed from observations on the behavior and reactions of relatively simple substances. Nature does not order the interrelations of particulate units to meet the pedagogical necessities of propagandists of this or that school. The simple phenomena have both chemical and physical aspects, and part of the whole truth lies in each aspect."[21]

J. R. Marrack[22] gave an excellent review of both the chemical and the physical aspects of immune reactions and pointed out the fact that it is possible to distinguish two stages in immunity reactions: (1) specific combination of "determinant" groups with antibody, and (2) secondary reactions—precipitation, agglutination, etc. Many "determinative" groups lack chemically reactive areas, and immunological equivalence may be shown by groups chemically different, *e.g.*,



and



Marrack states: "Such a result can only be due to intermolecular forces and the specific character must be ascribed to (1) an appropriate distribution of polar fields on the determinant group and on the antibody; and (2) to purely spatial considerations, since the approach of a determinative group to a receptive site on the antibody may be prevented by an inert substituent which gets in the way (steric hindrance). These are the same factors as determine the specific selection of the molecules which are built into a crystal."

It may here be noted that if adsorbed molecules are polar, which is commonly the case, the pattern of the molecular "ends" adsorbed on the oppositely charged areas of a complex surface would tend to form a monolayer or plaque having the *reverse pattern* of the surface on the near side and a *duplicate pattern* of the surface on the off side.[23] Over 20 years ago Nellensteyn found that diamond will adsorb methylene blue but not succinic acid; but the reverse is the case with graphite, which differs from diamond only in the spacing of the carbon atoms. While the formation of pure crystals of simple compounds with exclusion of "foreign" molecules may take place by the selective and oriented specific "adsorption" of new particles at the various growing crystal surfaces, in the case of very large molecules or molecular groups, like antigens and antibodies, a "spot welding" of molecules at limited reactive areas may suffice to form aggregates strong enough and large enough to settle out. Since time is a factor here, the drop in kinetic activity which accompanies particle growth will, up to the zone of optimum colloidality, favor the proper adjustment of attractive areas to each other. Moderate agitation

may also be a help, as is the case in the Kahn test for syphilis.[24]

The simple physico-chemical principles involved in immunology extend to many other fields, as might be expected. F. R. Lillie[25] found that seawater in which sea-urchin eggs had stood for a while ("egg-water"), will agglutinate the spermatoza of the same species, though the agglutinization is spontaneously reversible, seldom lasting over an hour. The phenomenon is observed in all echinoids, in many molluscs, and in some annelids. The substance responsible is called fertilizin, and A. Tyler found[26] that it comprises part or more probably the whole of the gelatinous superficial coat of the egg, which swells and slowly disperses, but which can be quickly dissolved in acidified seawater of pH 3.5 to 4.5. "Egg-water" also activates sperms. As evidence of this fact Dr. E. E. Just demonstrated to me at Wood's Hole (1925) that if a small dish in which a female *Nereis* has been floating were rinsed out several times with fresh seawater and then filled, a male *Nereis* dropped in will immediately shed its sperm.

Fertilizin reacts with a substance termed anti-fertilizin which can be extracted from the sperm surface by acidified seawater, which is of interest in connection with the mode of development of the sex glands. Curiously enough, the surface of the egg from which the fertilizin has been removed also furnishes a substance showing the same behavior and immunological specificity as anti-fertilizin. Commenting on the fact that these substances react with one another in the specific and complementary manner of antigens and antibodies, Tyler suggests application of this notion to explain the auto-agglutination of bacteria and the production of bacterial antibodies.

"The finding of two mutually complementary substances leads to the expectation that more may be found deeper within the cell. This view may then be expanded into a general theory[26] of cell structure; namely, that a cell is a mosaic of substances that are mutually complementary (*i.e.*, capable of combining with one another in the manner of antigen and antibody) substances which are actually in combination with one another in regions where they adjoin. The compounds would be represented by the various membranes, such as the cell membrane, nuclear membrane, nucleolar membrane, vacuolar membrane, etc., which in turn keep the complementary substances apart."

This view is consonant with the establishment of interfacial films, which, as the work of Harkins, Langmuir, Adam, Freundlich and others has shown, may be polymolecular and more complicated than

is indicated by the simple union of two oppositely charged ends of polar molecules. It also accords with the view of Alexander and Bridges that the cell is a "box-within-box" structure.

It must be remembered that every free unit or surface having unsatisfied residual electronic fields tends to draw to itself units of opposite charge pattern or areas of opposite electronic contour, even if several molecules are needed to complete the opposite mosaic. Crystallization proceeds this way with the elimination of stranger molecules; but it can also happen that there are "lacunae" or adsorbed "impurities," so that the film or layer, especially if a thick one, may not ideally perfect.

To go to one extreme, when atomic nuclei are deprived of one or more electrons, they strive to replace them or at least to share electrons with other nuclei, as in covalent compounds. At higher structural levels the residual forces become more feeble and indefinite, but nevertheless effective. Sir W. B. Hardy[27] showed how exceedingly difficult it is to secure a really clean surface, and we can understand how nascent atoms, molecules, and areas may be highly and specifically active, especially in the loci where they are liberated or formed. Lord Rayleigh pointed out that a film of "grease" adsorbed from the atmosphere exists on most exposed surfaces, and to this film all kinds of small particles of "dirt" may adhere. In South Africa when a slurry of diamond-bearing clay flows down a trough lined with tallow, the grease selectively fastens most of the diamonds. Microscopic examination of "house-moss" (or 'cobwebs")—that fluffy horror of meticulous housekeepers—shows it to consist mainly of tiny textile fibers (cotton, wool, etc.) with adsorbed "grease" and mineral particles. By twisting "house-moss" between the fingers, it can be "spun" into a weak but coherent "thread." And millions of letters daily travel in the mails with their postage stamps securely attached, though not even a strict stoichiometrist among chemists would suggest that the "compound" should be called stampate of envelope, or envelopate of stamp.

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[13] See J. Alexander, "Colloid Chemistry," 4th ed., p. 385, D. Van Nostrand Co., 1937.

[14] *Science*, **80**, Oct. 5 (1934).

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[17] The authors say: "In plating X-multilayers it has been observed that after about 500 layers have been deposited, and the electrostatic repulsive field of the multilayer has thus reached a certain value, the upper portion of the submerged probe has a silvery appearance which gradually moves down the probe with increasing number of dips, and film does not adhere to the multilayer over this portion. The electrostatic repulsive field thus sets a limit to the thickness of X-multilayers on insulators." Dr. Goranson informs me (private communication) that "if plating is made on a metal, more layers can be put on because of the oppositely induced charge of the metal."

[18] *Science* (1938), **87**, 510.

[19] *Brit. J. Exptl. Path.* (1935), **16**, 116.

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[21] "Colloid Chemistry," 4th ed., p. 418, by J. Alexander, D. Van Nostrand Co., N. Y., 1937. Those interested in a historical account of the long conflict between proponents of the various views and theories will find much summarized in "The Chemical Aspects of Immunity" 2nd ed., 1929, by H. Gideon Wells, and in earlier publications. More recent views are given by Wm. C. Boyd in "Fundamentals of Immunology" (Interscience Pub., 1943) and in his paper on Immunology in "Colloid Chemistry," Vol. V, pp. 957-979, Reinhold Publishing Corp., 1944. M. G. Sevag in "Immuno-catalysis" (C. C. Thomas, 1945) has also adopted the catalytic view.

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Chapter 8

Genetics: The Heritable Transmission of Catalysts

The Nobel prize in medicine for 1935 was awarded to Thomas Hunt Morgan, the outstanding geneticist, who with his able collaborators, Calvin B. Bridges, A. H. Sturtevant and others, did so much to establish experimentally the view that hereditary characters are carried by particulate units called genes (or gens),* arranged linearly in the chromosomes of the germ cells. This award to Morgan called attention to the great importance of developments in the science of *genetics*, a branch of biology dealing with heredity and its mechanisms.

While breeders of cattle, horses, dogs, pigs, fowl and other domestic animals, as well as farmers and seedsmen dealing with vegetables, wheat and other grains, had from time out of mind been making selections of favored forms, it was Gregor Mendel, Abbot of Brünn (Bohemia) who introduced atomism into genetics, as J. B. S. Haldane puts it. In his cloister garden, Mendel assembled the results of breeding experiments with the common garden pea (*Pisum sativum*), which were published in 1865, only six years after the appearance of Charles Darwin's "Origin of Species." Mendel attributed the differences, which he found were distributed by heredity, to discrete representative factors or producers, which in general pass unaltered through successive generations, though their distribution may vary. It was not until 1900, eighteen years after Mendel's death, that the importance of his work was independently recognized by three distinguished botanists, Hugo de Vries (Holland), C. Correns (Germany) and G. Tschermak (Austria).

Mendel crossed a *tall* with a *dwarf* pea, and observed how these characters were inherited. All members of the first family (F_1) were tall; but this was not the case with the second family (F_2) developing from the seeds of the F_1 hybrids.

* The Standard Dictionary of 1913 gives timidly the following definition of "gen": "A minute hypothetical particle supposed to be the bearer of hereditary qualities."

Mendel's First Law: Segregation

The F_2 families always gave an average ratio of three talls to one dwarf. The F_2 dwarfs always bred true to type, and so did one-third of the talls. The other two-thirds of the talls behaved just like the original F_1 hybrids; that is, they gave, in F_3, three talls to one dwarf. Mendel termed tallness a *dominant* character, and dwarfness a *recessive* character, since it reappeared in subsequent generations raised from the hybrid seed. Several other pairs of contrasting characters gave similar results, which are shown in the following diagram, where R indicates plants with a recessive character, D dominants which breed true, and *D* dominants which carry the recessive particular producer or gene, as it is now called:

Parents		D×R		
		↓		
Family F_1		*D*		
		↓		
Family F_2	D	*D*	*D*	R
	↓	↓	↓	
Family F_3	D	D *D D* R	D *D D* R	R

Although each cell of the hybrid F_1 evidently developed under the joint influence of a dominant and a recessive gene, in both the ova and the pollen grains these factors separate cleanly from each other, or *segregate,* so that, according to the laws of chance, each ovum or pollen grain would get a pure dominant gene, D, or equally often a pure recessive gene, R. Thus with random or haphazard fertilizations, we would get

Pollen	D	R
Ova	D	R

leading to an F_2 family averaging D/D; 2DR; R—that is, three dominants to one recessive.

Mendel tested this hypothesis by what is known as a *backcross*: he fertilized *dwarf* flowers with pollen from F_1 hybrids, and obtained, as he expected, *equal* numbers of tall- and dwarf-producing seeds. The following diagram indicates what happened:

Pollen of F_1 hybrid (tall)	D	R
Ova of recessive dwarf	R	R
Progeny of backcross	D/R, D/R 2 tall	R/R, R/R 2 dwarf

Mendel's Second Law: Independent Assortment

When peas having *yellow and round* seeds were crossed with peas having *green and wrinkled* seeds, Mendel found that all the F_1 seeds were yellow and round, these characters being dominant over the recessive alternates, green and wrinkled. But the plants from these F_1 seeds, when self-fertilized, gave seeds, and therefore progeny, of *four* types: yellow/round; green/wrinkled; green/round; green/wrinkled, in the ratios 9:3:3:1. To explain these results Mendel assumed that the segregation of the genes of the yellow/green pair takes places independently of the segregation of the round/wrinkled pair; so that there would be four equally numerous kinds of ova and four equally numerous kinds of pollen grains, which, uniting at random during fertilization, would give sixteen different combinations that yielded (bearing in mind the factor of dominance) *visible* types in the 9:3:3:1 ratio actually found.

Linkage

Soon after the resurrection of Mendel's work, Sir William Bateson and R. C. Punnett of Cambridge (England), in making experiments with sweet peas, found that the factor pairs (red vs purple flower) and (long vs round pollen grain) did *not* assort at random. When (red) and (long) entered a cross together, they tended to remain together in subsequent generations to a greater extent than warranted by random assortment. This failure of random assortment is understandable on the assumption that the genes of the two pairs involved act as discrete units or gene blocks in the *chromosomes.* Chromosomes are microscopically resolvable bodies in the cell; they contain invisible genes arranged in linear order. Chromosomes were well known to cytologists, but their importance in genetics became evident in 1907 when Frances E. Lutz, working with the evening primrose, *Oenothera* (the experimental plant used by H. de Vries), found a great variation in the the number of chromosomes in the various varieties, as follows: *O. lamarckiana,* 14 (its variety *gigas* has 28); *O. lata* and *O. albida* have each 15 chromosomes. Gates independently published some of these results a few months later.

In 1901 Charles E. McClung of Princeton discovered unequal chromosomes in the two sexes, a fact brought out in Figure 18, indicating how the chromosomes behave in the formation of sperm

and ovum, and the union of these units as the initial step (conception) in the formation of an individual. Besides the single pair of sex-chromosomes which determine the sex of the individual, there are a number of *autosomes* ("ordinary chromosomes"), which in man number 46 (23 pairs). In mammals, including man, female cells have additionally two *x*-chromosomes (female determiners), while male cells have additionally one *x*- and one *y*-chromosome.

In ordinary cell duplication, termed *mitosis*, the chromosomes

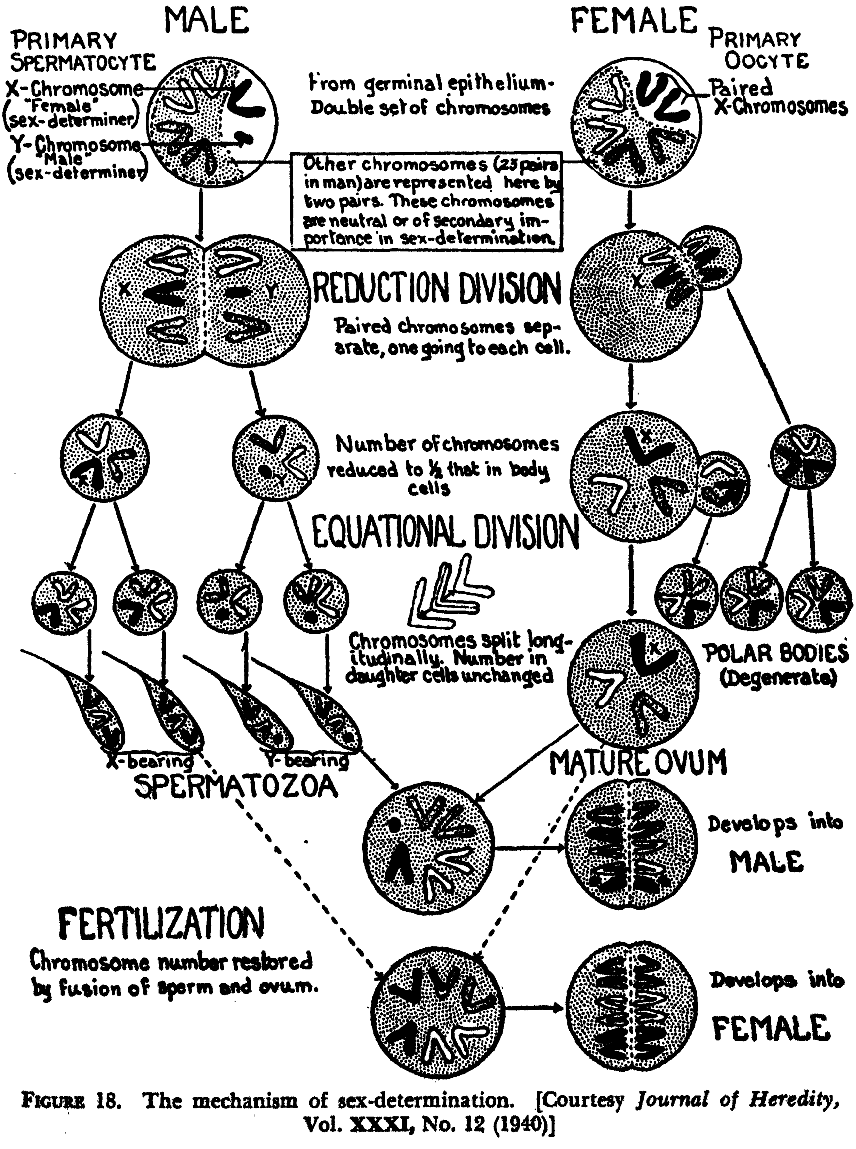


FIGURE 18. The mechanism of sex-determination. [Courtesy *Journal of Heredity*, Vol. XXXI, No. 12 (1940)]

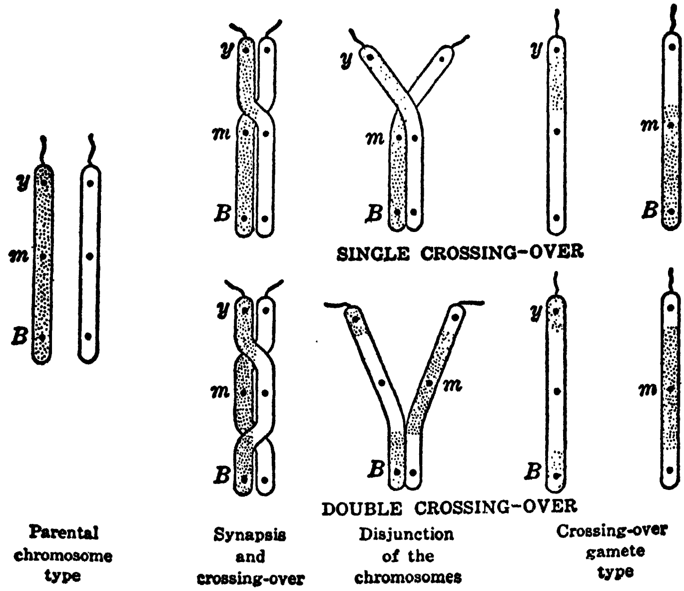


FIGURE 19. (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

duplicate themselves and the side-by-side partners separate, one set going to each of the two cells formed when the original cell divides. However in *meiosis*, the process of formation of the sex cells or *gametes*, the members of each chromosome pair separate, and the chromosome number in the resulting daughter cells is reduced to half the number in the somatic, or ordinary body cells. In 1909, Janssens (a Jesuit) in investigating meiosis, discovered what he called *chiasmata* (from the Greek letter X, *chi*) involving

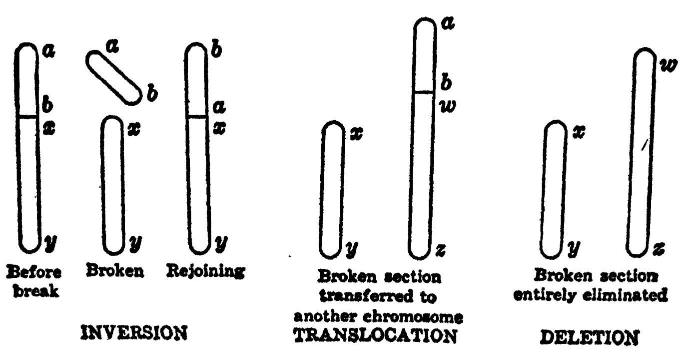


FIGURE 20. (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

exchanges of substance between maternal-derived and paternal-derived chromosomes, and thus establishing the cytological basis of the phenomenon of *linkage*. The four gametes formed by the extra (equational) division following upon reduction division, are thus usually different.

Variations in Chromosomal Behavior

The commonest of these variations is *crossing-over*, illustrated in Figure 19, which occurs when two chromosomes of a pair twist around each other in some stage of germ cell formation (meiosis). Less common forms are illustrated in Figure 20: *inversion*, when a block of genes breaks loose from a chromosome and returns to that same chromosome "upside down"; *translocation*, in which a loose gene block joins another chromosome; *deletion* or *deficiency*, in which the loose gene-block is lost. Heritable variations produced by x-rays often involve chromosomal variations.

A great impetus was given genetics in 1910, when Professor T. H. Morgan and his school began work with a tiny yeast-eating "fruit fly," which matures in about 10 days and lives only about a month (*Drosophila melanogaster*). About thirty or more prolific generations can be cheaply raised in a year, in quart milk bottles in the laboratory, quite independently of weather conditions. The breeding and crossing of strains is readily controlled. There are many recognizable variations, and a biologist can live long enough to make large numbers of "Mendelian" experiments, not possible where the life cycle is long or the progeny few. With elephants the gestation period is 22 months, and elephants do not produce litters used to count Mendelian averages.

From the strength of linkage between genes, it became possible to construct a map of the chromosomes indicating the relative position of many genes. Figure 21 is a "gene map" prepared by C. B. Bridges; the practical meaning of a few of the characteristics identified in the "shorthand" of geneticists, is shown in Figure 22.

A large variety of plants and animals, including microorganisms, are now being used in genetic experiments, and besides the *visible* characters or differences which so readily meet the experienced eye, many latent or hidden chemical differences are being explored. Thus by raising microorganisms in media with carefully controlled chemical content, it has been possible to learn something of the synthesizing properties of their genes, as shown in the work of Prof. George W. Beadle and collaborators of Stanford University with the red bread mold *Neurospora crassa*.

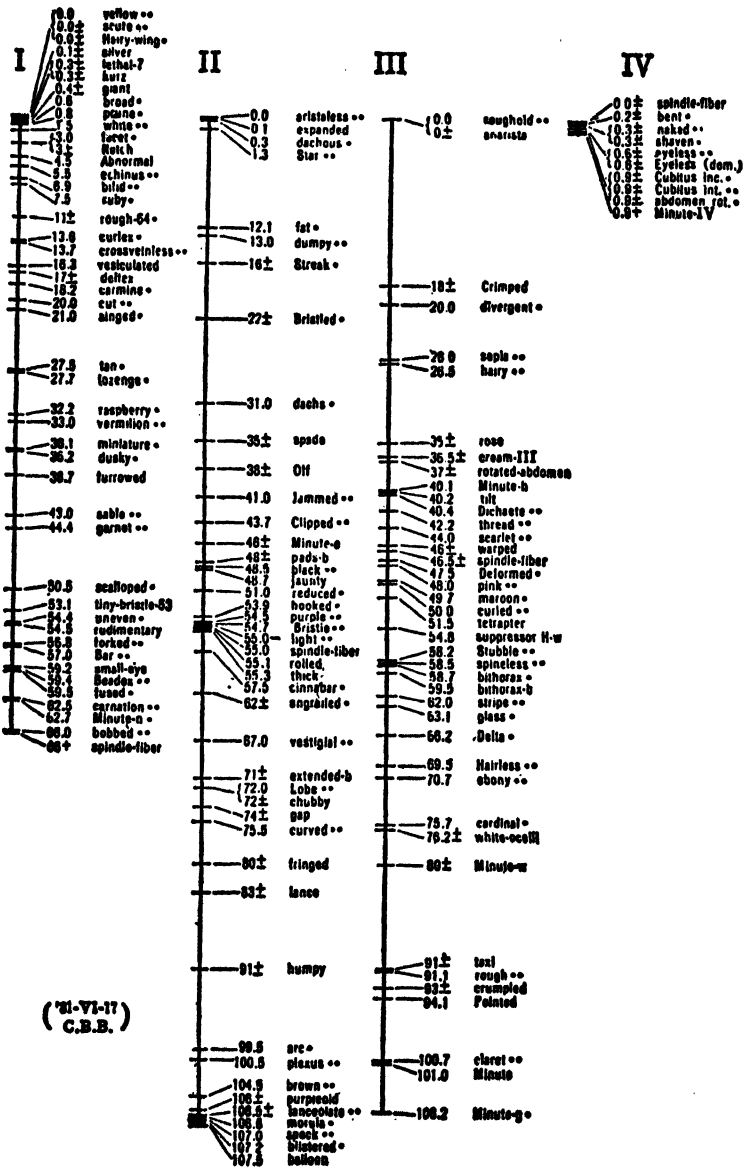


FIGURE 21. Genic map of the four chromosomes of *Drosophila melanogaster*, showing the linear order and distance apart of the genes (*after Calvin B. Bridges*). (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

The two mating types or sexes of this mold are morphologically indistinguishable, but they may be cultured indefinitely by asexual multiplication if kept separately, though when put together under suitable conditions mating and sexual reproduction occur. Unlike the higher plants and animals which are *diploid* (*i.e.*, their chromosomes occur in pairs), *Neurospora* is a *haploid* organism (*i.e.*, its seven chromosomes are singletons), which simplifies matters genetically.

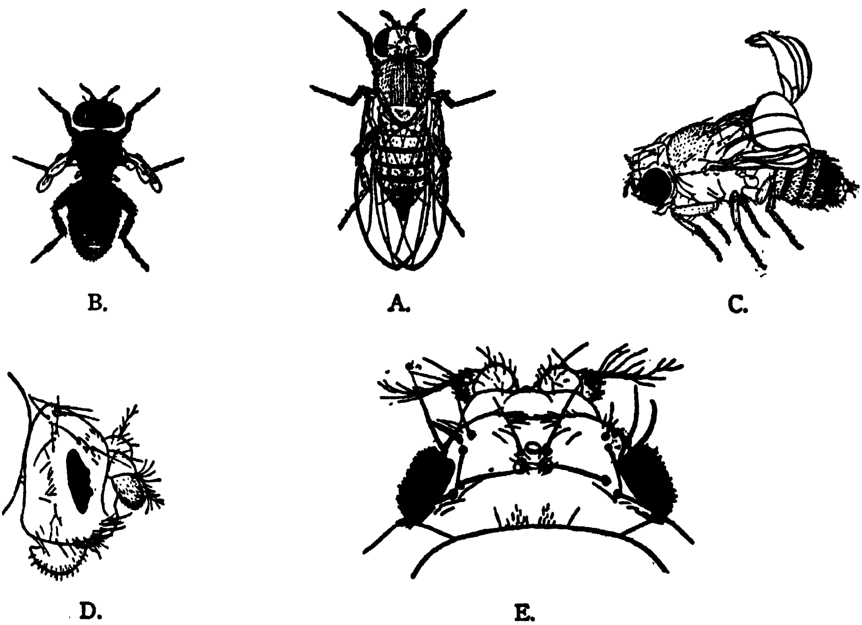


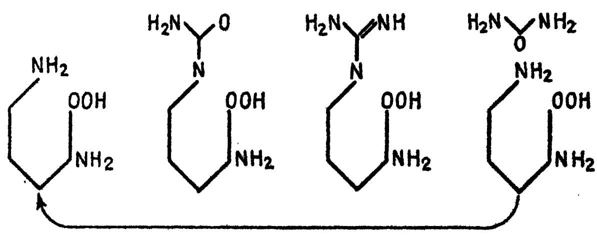
FIGURE 22. A. Normal wild-type female *Drosophila*, gray body color, red eyes, long wings, absence of speck at base of wings. B. Male *Drosophila* having four linked, recessive characters, viz., black body color, purple eyes, vestigial wings, and speck at the base of the wings. C. Fly showing the mutant character "Curly." (The wings curl up at the ends and are held somewhat apart.) D. Eye of female fly homozygous for "Bar" (eye is bar-shaped). E. Fly showing the mutant character "Lobe." (The eyes are small and protruding.) (Courtesy "The Theory of the Gene" by Thomas Hunt Morgan, Nobel Laureate. Yale Univ. Press, 1926.) (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

When a sexual diploid is formed by the fusion of two individuals of opposite sexes, it does not multiply as such, but immediately forms haploid spores which are the equivalent of gametes. As Beadle states:[1] ". . . all the products of the nuclear divisions which reduce the chromosome number from the double number 14 to the single number 7 can be recovered in sexual spores and cultured. The result of this is that the offspring of parents different by one gene will occur in a mechanically fixed ratio of one-to-one. We can omit the phrase 'on the average' which has to be applied to genetic ratios observed in an organism like man."

Considering its simpler needs, *Neurospora's* synthesizing catalysts enable it to make its needed amino acids and vitamins from simpler chemical compounds. By the use of standard bacteriological technique the mold can be kept uncontaminated on a "minimal" medium containing water, certain inorganic salts, sugar or some other source of carbon and energy, and a minute amount of biotin, a vitamin of the B complex.

In 1927, H. J. Muller (Nobel prize, 1946) made an outstanding advance by showing[2] that heritable changes may be induced in animals and plants by treating their sex cells with x-rays of selected nature; and since then a huge literature has developed. This was at first called the "artificial transmutation of the gene," but it is now known that chromosomal breakage is often involved. Assuming that *Neurospora* has genes which govern the production of the chemical substances needed for its life, Beadle bombarded sexual spores with ultraviolet or x-ray photons, and then placed the treated spores on media to which various growth factors had been added. If a treated strain could grow on the special medium but not on the "minimal" medium, it was obviously deficient in catalysts capable of making the substance added to the fortified medium. Thus several dozen mutant strains produced in *Neurospora* by irradiation are unable to synthesize the amino acid arginine from the minimal medium, though normal *Neurospora* can do so. These mutants thrive only when arginine is supplied.[3]

In mammals, arginine is formed in the so-called ornithine cycle of Krebs and Henseleit.[4] *Neurospora* appears to make arginine in the same way, for some mutant strains cannot convert citrulline into arginine, and therefore require arginine; others cannot convert ornithine into citrulline, and therefore require citrulline or arginine; still others cannot synthesize ornithine, and therefore fail to grow in the absence of ornithine, citrulline or arginine. Beadle states: "The interesting thing about this from the standpoint of gene action is that we find just what would have been predicted from the hypothesis that enzymes are dependent upon specific genes and that for each enzyme there is a specific gene, and *vice versa*. In each instance of defective



Ornithine *Citrulline* *Arginine* *Ornithine + urea*

arginine synthesis the genetic study shows a modification of one gene."

Tryptophane, an amino acid essential for humans, is made by *Neurospora* by condensing serine with indole. The catalyst deficiencies of certain *Neurospora* mutants prevent them from making indole; but if indole or tryptophane is supplied, they form in the medium anthranilic acid, the precursor of indole. Still another mutant is unable to synthesize anthranilic acid, but can convert it into indole. "Each strain differs from the original wild-type *Neurospora* by a single gene, and the two mutant genes concerned can be shown to lie in different positions in the chromosomes. . . . In the future we may

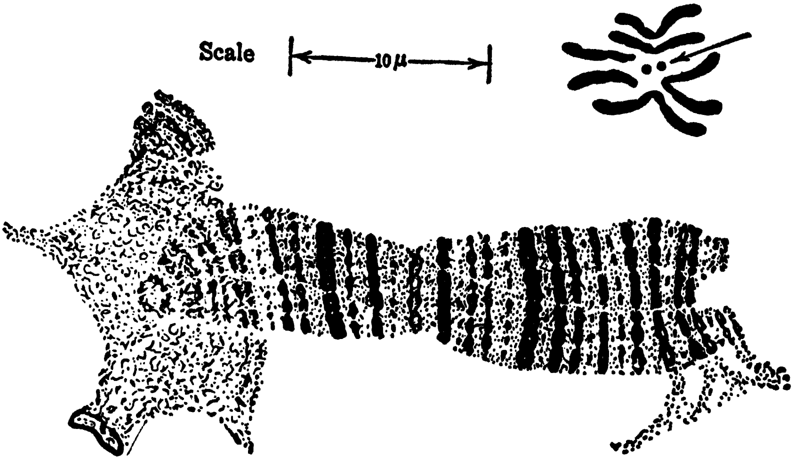


FIGURE 23. Salivary gland chromosome from *Drosophila melanogaster.* (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

expect to see increasing use of genetics this way by biochemists." Choline, a carrier of methyl groups to other molecules, and a component of the important protoplasmic constituent lecithin, is made by first synthesizing a precursor, monomethylethanolamine and then adding two additional methyl groups.

The Salivary Gland Chromosomes

In 1935 Professor T. S. Painter of the University of Texas examined the cells in the salivary glands of the tiny grub representing the larval stage of *Drosophila,* and made the important discovery that in these cells the chromosomes are swollen from 175 to 200 times their length in ordinary cells. In the extended chromosomes there are great numbers of characteristic bands, which vary with and correspond to the genetic constitution of the individual grub, and therefore constitute a "genetic spectrum."

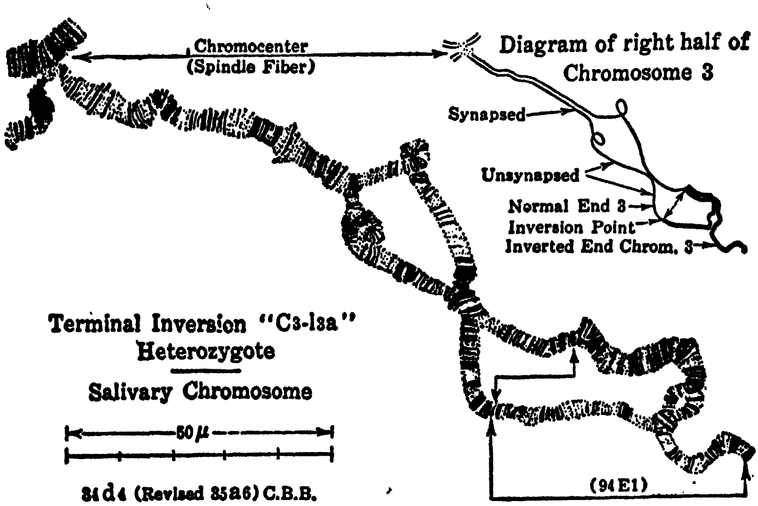


FIGURE 24. (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

Figure 23, on which the scale of magnification is indicated, shows in the upper right-hand corner the four pairs of autosomes and the one pair of sex chromosomes as usually seen in cells of *Drosophila*. The main portion of the figure, at the same magnification, shows large size and the wealth of details revealed in the tiny sex chromosomes as found in a salivary gland cell. Figure 24 illustrates a case wherein a terminal inversion in chromosome 3 has prevented complete synapsis of the chromosomes. Figure 25 illustrates a portion of chromosome 2 where a deficiency (lack of a gene block) has caused an outward looping of the normal chromosome during conjugation. Figure 26 shows a portion of salivary gland chromosome 2 in which the data of the gene maps

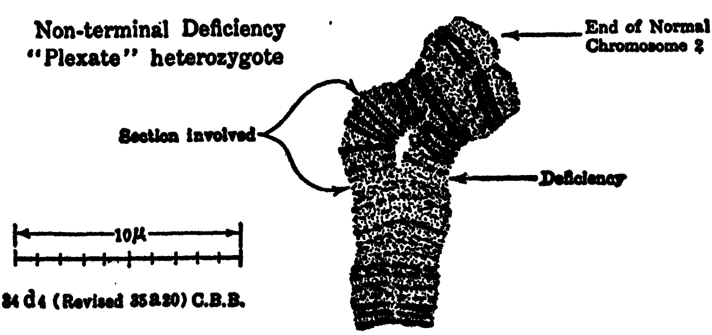


FIGURE 25. (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

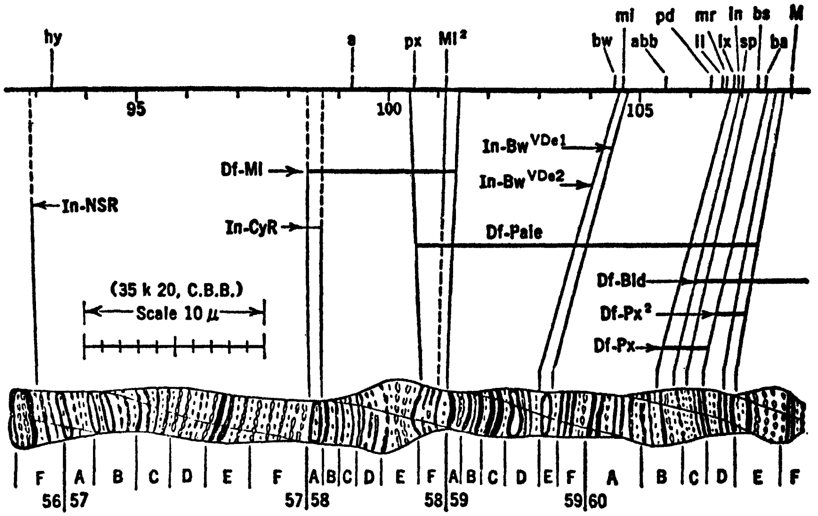


FIGURE 26. Section of the right end of second chromosome (salivary gland of *Drosophila melanogaster*) with corresponding portion of the chromosome map (*after Calvin B. Bridges*). (From paper in "Colloid Chemistry," Vol. 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

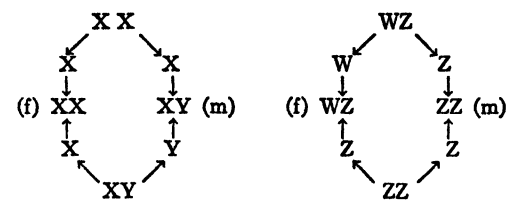
finds visual confirmation in the "genetic spectrum" there revealed. Many more complete concordances are shown in the elaborate salivary chromosome maps recently published in the *Journal of Heredity*. Figure 27 outlines the 48 human chromosomes (24 pairs).

Sex Determination

In contradistinction to the main number of autosomes are the usually smaller "sex chromosomes," so called because their segregation determines the sex of each offspring. The basic sex chromosome, known as the *X*-chromosome, has no discovered mate in some species (*XX-XO* types). Only comparatively recently (1901) was the *Y*-chromosome found in human sperm, placing man in a major *XX-XY* group in which the fertilized ovum that develops

FIGURE 27. The 48 human chromosomes in outline (*after H. M. Evans and O. Swezy*, Mem. Univ. Cal., Vol. 9, No. 1929). (From paper in "Colloid Chemistry," Vol 5, by J. Alexander, Reinhold Publishing Corp., N. Y.)

into a normal female has two *X*-chromosomes, whereas the normal male has one *X* and one *Y* chromosome. In man during meiosis (including reduction-division) each female gamete (ovum) is left with one *X*-chromosome, while the sperms are equally divided between *X* and *Y*; therefore the sex of the off-spring is determined by which kind of sperm happens to fertilize the ovum. With another group of animals, including birds and moths, it is the female that has the differing sex chromosomes (*WZ*), the male cells (*ZZ*) yielding only *Z* sperms. The following diagrams indicate how, in these two groups, these microscopic gene-carrying units dominate sex determination:



In sex determination, as in all other cellular developments, *all* the genes exert an influence. The net results which emerge (only some of which can be demonstrated) are dependent upon the final results of all the very complex and interrelated cellular catalysts and their physiological and morphogenic consequences, a summation of all this being included in the expression *genic balance*. The biont (plant or animal) may die or fail to reproduce if genic balance is too greatly disturbed—in fact, most mutations are lethal.

In certain abnormal cases chromosome pairs fail to separate during reduction-division in meiosis, a phenomenon termed *non-disjunction*. As a consequence abnormal numbers of chromosomes may enter into a gamete (germ cell) and hence into a zygote. With *Drosophila*, some of the consequences are tabulated below, *A* representing a single set of autosomes. Each parent contributes one autosome set, under normal conditions.

AAXY (normal diploid fly)	Male (normal
AAXX (normal diploid fly)	Female (normal)
AAXXY	Female, giving abnormalities.
AAXXX	"Superfemale," abnormal
AAXO	Male, sterile
AAYO	Dies (sex?)
AAAXXX (triploid fly)	Female, producing abnormalities

AAAXX	Intersex, sterile
AAAXXY	Intersex, sterile
AAAXY	"Supermale," sterile

It must not be supposed, however, that heredity is carried by the genes alone, or even solely by the chromosomes in which x-ray treatment may produce "position effects" having consequences as marked as "point mutation." In Chapter 9, these matters are discussed at length in connection with differentiation and morphogenesis. However, we may here refer to a few other non-genic and non-chromosomal factors.

First are the so-called "maternal effects," observed for example in the mode of coiling of the shell of the snail *Limnaea peregra*; the direction of coiling (righthand or lefthand) depends in the F_1 generation upon the genotype of the mother, not upon the genotype of the individual (Sturtevant, 1923). The work of Toyama (1912) with silkworms (Bombyx mori) indicated that the appearance of an egg (shape, color) depends not upon its own genetic constitution (maternal and parental genes), but upon the genes of the mother in whose body the egg developed.

Definite cytoplasmic inheritance is illustrated by the self-reproducing plastids, *e.g.*, chloroplasts which largely dominate photosynthesis in plant cells.[5] Wanda K. Farr has shown[6] that cellulose is formed in a chloroplast or colorless plastid. Starch granules likewise develop by deposition of material from the plasma of certain plastids around small starch-forming centers.

In the case of variegated plants, the leaves show whitish spots or stripes where the cells are devoid of chlorophyll generally because viruses (infective agents approaching molecular dimensions) destroy the chlorophyll or prevent its formation. These viruses, and therefore their effects, may be heritably transmitted; variegation apparently does not harm the plant and is regarded as a desirable feature by nurserymen. On the other hand, some viruses produce very undesirable effects, *e.g.*, influenza, poliomyelitis, foot-and-mouth disease, yellow fever, chicken sarcoma (Rous), rabbit papilloma (Shope), encephalitis. But though these may be transmitted by some form of infection or inoculation, and may lead to formation of specific antibodies, they are not effectively transmitted by heredity. However, we do not know to what an extent harmless or beneficial virus-like symbionts or "diseases" may be carried by heredity, as is the latent potato mosaic virus which shows its effects only in susceptible strains of potato.

But in many plants plastid color is gene-determined, and is transmitted on a Mendelian basis.[7] Most geneticists express the Scotch verdict of "not proven" on experimental work supposed to demon-

strate cytoplasmic inheritance, even though V. Jollos produced "*Dauermodifications*" in which cytoplasmic effects persisted through hundreds of generations, and V. von Wettstein was able to keep some hybrid mosses for fifteen years without indication that their cytoplasm had been gradually reconstructed by foreign genes. Wettstein classified components of the germ plasm (cytoplasm plus all particulate units) as follows: (1) the genome, *i.e.*, all genes carried by the chromosomes; (2) the plastidome, arising from the properties of the plastids themselves; (3) the plasmon, genetically effective portions of the cytoplasm.

Where prolonged and skillful experiments fail to secure a desired result, the feeling grows that "it can't be done." Such a despairing note is sounded by calling viruses "obligate parasites," when the truth simply is that we have as yet been unable to find a non-living medium for them. However, some kinds of viruses are now cultivated on chorio-allantoic membranes of developing hen's eggs, on a commercial scale for use in immunization. The viruses of poliomyelitis and of the foot-and-mouth disease do not grow on these membranes.

Professor E. G. Conklin stated:[8] "The classic argument of the Weismannians was that *we can conceive of no mechanism* by means of which somatic changes can be carried back into the germ cells, *and therefore there is no such mechanism.* Now the fallacy of this argument is obvious; even if we could conceive of no suitable mechanism for this purpose, this does not preclude the existence of such a mechanism." As the all-pervasive phenomena of catalysis became more and more recognized and understood, it became evident that in catalysis we have what still seems to be the only basic mechanism whereby we can explain the underlying chemical changes which directly or indirectly govern life and its physical manifestations. Catalysis discloses the required mechanism, which is not revealed by valid views of what happens at higher structural and organizational levels, so that the very terms used in expressing these views imply results rather than mechanism. As Thistleton Dyer remarked in his address to the British Association for the Advancement of Science at Bath in 1888: "Science will always prefer a material *modus operandi* to anything so vague as a tendency."

Everyone recognizes that the cytoplasm is a specialized milieu in which and with which the genes and other inclusions function. Chapter 9 considers the importance in differentiation of the mil-

lions of specific molecules included in the cytoplasms of sperm and ovum. Many other kinds of specific atoms and molecules are in most cases essential to full growth, development and reproduction, *e.g.*, numerous vitamins and trace elements. These substances commonly serve as prosthetic or activating groups in essential biocatalysts which have a more or less limited functional life and must therefore be continually renewed.

What happens if strange and unusal atoms or molecules find their way into the cytoplasm? While no unequivocal answer can be given, in most cases where the invading substance is not destroyed or utilized, it would probably be lethal. Certain constituents of venoms or toxalbumins like ricin, abrin, botulinus toxin, seem to function directly or indirectly as catalysts* and to produce extensive chemical breakdown entirely out of proportion to their small mass. It is certainly reasonable to expect that in some cases stranger molecules may produce effects which are beneficial, either by modifying existing catalysts or by serving to create new ones. From the standpoint of genetics the important question is: Can these new catalysts be carried on by heredity? Experimental evidence is accumulating to show that they can, thus establishing a physicochemical basis for a mitigated form of Lamarckism, which has been taboo in biological texts and teachings because of lack of experimental evidence.

Of particular interest here is the work on the ciliated protozoan *Paramecium aurelia* by Professor T. M. Sonneborn of Indiana University, who was awarded the prize of the American Association for the Advancement of Science on Jan. 1, 1947. One race of variety 4 of this organism makes the fluid in which it lives poisonous for nearly all other races of *Paramecium*, and is therefore known as a "killer"; but it is resistant to its own poison. The "killer" character is determined by a four-component system:

(1) a nuclear gene, *K*
(2) an active product of this gene
(3) a cytoplasmic unit, known as *kappa*
(4) a final poisonous product or substance, called *paramecin*

The paramecin produced by one "killer" race may kill animals of another "killer" race; in one instance, two "killers" reciprocally killed each other. At least one "non-killer" race was found resistant to the paramecin produced by certain "killers"; but as a rule "non-killers" are "sensitives"—that is, they are affected by the killer poison.

Though no gene or genes can alone initiate the production of

* Or catalyst inhibitors or "poisons".

kappa, gene *K must* be present if *kappa* is to be maintained and multiplied in the cytoplasm, because if *K* is replaced by its allel *k*, *kappa* disappears within a few cell divisions and the progeny become permanently sensitive to paramecin. However, gene *K* cannot alone, when introduced into a cell that has lost *kappa*, re-establish *kappa* formation. But if a small amount of *kappa* is introduced into sensitive *KK* stocks, then *kappa* is maintained and multiplied in all the progeny. This Sonneborn terms "primer" action, and notes its analogy to the finding of Cori in phosphorylase studies, that the presence of *some* glycogen is apparently necessary to the formation of more glycogen. A reasonable but unproved explanation is that gene *K*, modified by or under the influence of *kappa*, molds off or produces something (molecule or molecular group) which serves as an enzyme, or else that *kappa* forms a cytoplasmic catalyst, with some product of *K*, the net result of the new catalyst area being the production of paramecin and of *kappa*.

The macronucleus of *P. aurelia* is a compound nucleus containing at least 60 *K* genes. Several experimental methods indicate that about 250 *kappa* particles exist in the cytoplasm of a "killer" cell; but there appears to be much less paramecin than *kappa*. The paramecin is released into the medium very slowly, at any stage of the five-hour fission life cycle.

The rate of *kappa* multiplication varies in different stocks, and is affected by conditions, temperature being important. When the concentration of *kappa* is somewhat reduced experimentally, paramecin is no longer formed, but the cells remain resistant to its action. Further reduction of *kappa* lowers resistance to paramecin; but as long as a single particle of *kappa* remains in a cell containing gene *K*, the concentration of *kappa* can be increased by appropriate conditions. As *kappa* concentration increases, the sequence of phenotypic types is reversed, sensitivity to paramecin disappears, and the cells become resistant. With further increase in *kappa* concentration, production of paramecin begins again.

On cell division, *kappa* is unequally divided, and the studies of J. R. Preer show that this inequality corresponds precisely to chance or random distribution of *kappa* at the time of fission. Referring to the differences found in the Dionne quintuplets by J. W. MacArthur,[9] J. Alexander stated:[10] "The slight differences found in monozygotic twins do not seem to be due to the precisely duplicated genes, but rather to inequalities in the apportionment of the zygote cytoplasm upon mitosis, involving unequal distribution of catalyst modifiers between the cells, which later develop into separate individuals." Sonneborn points out that the concentration of *kappa* is of the order of magnitude of one particle per 1,000 μ^3, which suggests that the chemical laws of mass action, predicated upon probability calcula-

tions, may here be replaced by the laws of the action of single particulate units. And the formation of a new catalyst area can change the whole course of chemical events.

The experimental facts indicate that gene *K* and *kappa* cooperate in forming catalyst areas capable of synthesizing both *kappa* and paramecin, and that this heritable result is not exclusively genic.

Results of experiments on the chemical production of mutations, begun in 1940 at the University of Edinburgh, have recently been released.[11] Mustard gas, the diethyldichlor sulfide used in World War I, produces heritable changes in *Drosophila* and *Tradescantia* by causing chromosome breaks and rearrangements, and genic changes, in some respects like those produced by x-rays.[12]

Treatment with mustard gas produces a much larger percentage of sex-linked lethals than does x-ray treatment, and also a much larger percentage of "mosaics" among the mutated individuals. In one striking case "a son of a mustard-gas-treated male was, both in the gonads and in the soma, a mosaic for two different mutations of the same gene, although it must be assumed that in the treated spermatozoon each treated gene was present only once. An explanation which seems particularly satisfactory in accounting for all these observations is that the gene affected does not always mutate at once, but may acquire a tendency to mutate which remains latent until a later cell division. Support for this hypothesis was obtained when it was found in several cases that the offspring of gonadic mosaics for a mutation again were gonadic mosaics for the same mutation. In these cases an induced specific instability seems to have been transmitted from one generation to the next before giving rise to a stable change. No parallel observations have been reported in literature on radiation genics; but it seems worth noting that so-called unstable genes, *i.e.*, genes which tend to mutate repeatedly in the same direction, have been found several times in untreated material. Says D. E. Lea,[13] ". . . it is tempting to consider the possibility that one of the means by which evolution adapts mutability to environmental requirement is the achievement of a balance between the production of mutagens and sensitivity to them."

While this experimental evidence of cytoplasmic inheritance does not mean that any and all nongenic changes are necessarily heritable, it demonstrates the principle and compels us to regard it —to use a legal analogy—as permissive, though not mandatory in function.

In his book "Hormones and Heredity" (London, 1921) J. A. Cunningham suggested that the mechanism whereby "modifications produced in the soma by external stimuli could affect the

determinants in the gametes in such a way that the modifications would be inherited," involved the action of hormones; the latter were defined as special chemical compounds which take the place of the imaginary gemmules in Darwin's theory of pangenesis and of the "constitutional units" of Herbert Spencer. In stressing the view that the theory of the hereditary transfer of somatic modifications is not in conflict with the theory of genic mutations, Cunningham wrote: "... there are two kinds of variation in evolution, one somatic and due to external stimuli, acting either directly on passive tissue or indirectly through function, and the other gameto-genic and due to changes in the chromosomes of the gametes which are spontaneous and not in any way due to modifications of the soma. Adaptations are due to somatogenic modifications, non-adaptive diagnostic characters to gametogenic mutations. It is a mistake to attempt to explain all the results of evolution by a single principle."

Professor W. H. Manwaring of Stanford University[14] stated: "These studies suggest a Lamarckian rather than a Darwinian world, or at least a world in which both Lamarckian and Darwinian evolutionary mechanics are operative." (This refers to his review).

Henry Fairfield Osborn stated[15] in the third of a series of addresses on the origin of species, that the theories of Buffon (direct action of environment), Lamarck and Darwin, usually regarded as contradictory, are really complementary, for they all turn on the question of inheritance or transmission of individual adaptation, which may furnish the key to evolution. "On the affirmative side paleontology proves in the long run of geologic or secular time that both Buffon and Lamarck, as well as Darwin, were right in their main conceptions: organs starved by unfriendly environment finally disappear; organs which do not pay their way or are starved by disuse slowly drop out of the germ-plasm; vitally essential organs are either absolutely stable or progressive. Why not therefore concede the truth of the great conceptions of Buffon and Lamarck, even if immediate inheritance by the germ is disproved in the great majority of cases? Why not concede the still greater conception of Darwin, misled as he was to time by the marvelously rapid evolution of the germ-plasm witnessed in artificial selections?"

Professor Herbert S. Jennings[16] in speaking of the interaction of cytoplasm and chromosomes in genetics and in development, points

out that these two cellular divisions "are not separate in fact and in substance, though they are often kept isolated in concept—to the great detriment of sound understanding. There is a continual cycle of interchange between them, a transformation of material from one into the other . . . The cytoplasm is not merely passive; it actively determines what shall happen in the chromosomes . . . Every cell retains all the chromosomal materials, but which of these materials comes into action in each cell depends upon what cytoplasm is there present, as well as upon the conditions under which the reactions occur . . ."

In the case of the killifish (*Fundulus*), A. Richards[17] observed that the condensed chromosomes become vesicular by taking in cytoplasmic material and forming a vesicle hundreds of times the size of the original chromosome. These swollen chromosomes touch, press together, and form the nucleus. Then the vesicles discharge their content into the cytoplasm, including great numbers of chromatin particles detectable by cytological methods. The condensed chromosomes for the next cell division are formed from minute reserve parts of the large vesicles. Jennings states: "The pictures that we see of the condensed stages of chromosomes, at the time when they are undergoing division, are most misleading if they are assumed to show the distinctive characteristics of chromosomes. The chromosomes are active and changeable; they continually operate on the remainder of the cell (which we lump together as cytoplasm), visibly interchanging material with the cytoplasm, altering it, differentiating it . . ." Professor E. G. Conklin[18] summarized his similar observations on the common slipper-limpet *Crepidula* thus: "One might speak of these changes in the nucleus as systole and diastole, by means of which an exchange of nuclear and cytoplasmic material is brought about." But it must be remembered that the material discharged by the vesicles has been subjected to the influence of the local catalysts, including the genes and the enzymes arising from them.

Jennings then lists three classes of heritable modifications in protozoa: (1) degenerate changes induced by unfavorable conditions acting for many generations; (2) acclimatization to high or low temperatures or to injurious concentrations of chemicals (As, Sb, quinine, methylene blue and various organic substances); and (3) alterations in form and structure not involved in (1) and (2), which may be retained for hundreds of generations. Referring to the work of G. F. De Garis[19] on *Paramecium caudata* through a long series of asexual generations, Jennings remarks: "At every succeeding fission the original cytoplasm is diluted to one-half, so that after ten generations it is diluted to less than 1/1000 part, the remainder being new cytoplasm produced by growth. Yet after ten generations the original cytoplasm still has a marked effect on size. The original cytoplasm seemingly must therefore have to some extent the power of reproducing itself

in its distinctive nature, at the time that growth occurs. In this respect it partakes of the character of a gene or genetic material, in that it affects the character of the individuals and reproduces itself in some degree true to type. But in time it is made over by the nucleus."

When a race of *Paramecium* averaging 198 microns in length was mated with another true-breeding race averaging 73 microns, the influence of the cytoplasm was evident in one experiment for 36 generations, for only then did the descendants reach about the same size, even though they all had the same chromosomal content. Referring to experiments of V. Jollos,[20] which indicate that such environmental modifications as acclimatization may be inherited for as much as 800 generations, Jennings says: "If they are merely modifications of the cytoplasm one might anticipate that long before so many generations had passed the cytoplasm would have been made over by the nucleus, and its modifications would have disappeared. Yet we do not know that the time required for the nucleus to dominate the cytoplasm would be subject to the same limits in all cases. The question whether inherited environmental modifications are exclusively cytoplasmic, or whether they affect the nucleus, the chromosomes, must be left open for the present."

It is fortunate that heritable nuclear, genic, symbionic, and cytoplasmic changes are the exception rather than the rule, for otherwise nature would present a confused Babel of bionts. Before any change can be passed on by heredity, the catalysts or other precursor units involved must themselves be able to run the catalytic and chemical gantlet of the organism and not only survive but also be duplicated. Furthermore, the consequences to the organism, stemming from these heritable changes must be advantageous (and thus possible steps in evolution), or else harmless or not too detrimental.

As a counterbalance to the slight probability of the emergence of a markedly advantageous heritable change, we must remember that nature is continually conducting vast multitudes of experiments of this kind, over endless eons, and under an enormous range of ever-shifting conditions. But even where a newly developed individual is of a type capable of persisting and of dominating under conditions existing at the time of its emergence, it would still have to face the possibility of being overwhelmed by established forms and predators.

However, each new form of biont which becomes established tends to add to the amount and especially to the variety of chemical substances available to other bionts, either through the prod-

ucts of its metabolism or through its own body, living or dead. In general, each higher form of life depends upon simpler forms to supply it with essential molecules, either for the main mass of its food (fats, carbohydrates, proteins), or for many trace substances (*e.g.*, vitamins, minerals), largely needed to make up the catalytic machinery of the body, whereby the food molecules are molded into its own species-specificity through the analytic and synthetic action of its own catalysts. All this shows the truth of the French aphorism: "*Rien n'est la proie de la mort; tout est la proie de la vie.*" (Nothing is the prey of death; everything is the prey of life.)

Apart from the obvious fact that the chemical nature of "food" has thus an important evolutionary aspect,[21] we may refer to symbiosis and parasitism, in which bionts live in very close association. Many parasites (*e.g.*, tapeworms) pass part of their life cycles in intermediate hosts. The wood-eating termite would starve to death on this diet, were it not for the fact that it harbors an intestinal messmate (an endameba) whose catalysts can convert the wood into substances which the termite can utilize as food. Cleveland found that slight increase in oxygen pressure kills the endameba without harming the termite, but the termite then starves to death on a diet of wood.

Only recently (since about 1870) symbiosis was first discovered in lichens, which consist of an association of a higher fungus with a unicellular or filamentous alga. The fungus supplies water, salts, nitrogenous substances and "lichen acids" which slowly attack rocks, while the alga synthesizes carbohydrate; so that the partnership can succeed where either member would fail. Not only have lichens been "synthesized" from suitable fungus and alga, but small lichen-thalli have been grown on nutritive solutions in the absence of their algal constituent. The culture of orchids from seed became possible only after it was found that in nature a certain symbiotic fungus surrounds the seeds with special moisture and other conditions. Similar is the case of trailing arbutus, that delicate gem of the woodland in earliest spring.

Since there seems to be an ever-increasing amount of experimental evidence that some non-genic heritable changes actually are transmitted, we are justified in asking this question: How can heritable factors, apart from genic and chromosomal changes, enter into and affect the germ cells (gametes) through which heredity is transmitted?

A great variety of specific molecules and other particulate units are carried throughout every biont by diffusion, convection and circulation. In the course of time each cell, including the gametes, will receive whatever of this molecular flotsam and jetsam can enter into and endure in its cytoplasm, or else establish there specific catalyst surfaces, so that among these entering molecular or near-molecular units would be those that determine, or assist in determining, differentiation. Apart from other evidence, the fact that some differentiated cells have been found to breed true in tissue cultures warrants the belief that specific tissues or organs contribute to the molecular melée in the organism units capable of determining the formation of their own specific type of cell. On the other hand, de-differentiation may occur: heart cells tend to breed true as heart cells, cancer cells as cancer cells, etc.

Since excessive use of a part or of an organ commonly leads to its hypertrophy, this would tend to increase the total quantity of hypothetical modifier substances emanating from such part or organ, and would increase its equilibrium amount in the gametes. This is a hypothetical physicochemical mechanism which might in some cases lead to the inheritance of acquired characteristics based on the use or disuse of parts.

Without suggesting a mechanism for their action, J. T. Cunningham[22] advanced a Lamarckian view involving hormones as active factors in heredity. He pointed out that special glands are not essential for the production of these internal secretions; for his colleagues at the University of London, W. M. Bayliss and E. H. Starling, had shown in 1902 that the wall of the intestine produces the hormone secretin which, carried by the blood, activates the pancreas to secrete. Cunningham stated: "There is nothing improbable in supposing that a tissue stimulated to excessive growth by external irritation would give off special substances to the blood. We know that living tissues give off waste products, and that these are not merely pure CO_2 and H_2O, but complicated compounds. The theory proposed by me in 1908 was that we have within the gonads numerous gametocytes whose chromosomes contain factors corresponding to different parts of the soma, and that these factors or determinants might be stimulated by waste products circulating in the blood and derived from the parts of the soma corresponding to them. There is no reason to suppose that an exotosis formed on the frontal bone as a result of repeated mechanical stimulation due to the butting of stags would give off a special hormone which was never formed in the body before, but it would probably in its increased growth give off an increased quantity

of intermediate waste products of the same kind as the tissues from which it arose gave off before. These products would act as a hormone on the gametocytes, stimulating the factors which in the next generation would control the development of frontal bone and adjacent tissues. . . . If the factors in the gametes were thus stimulated they would, when they developed in a new individual, produce a slightly increased development of the part which was hypertrophied in the parent soma. No matter how slight the degree of heredity effect, if the stimulation was repeated in every generation . . . the heredity effect would constantly increase until it was far greater than the direct effect of stimulation."

Starling formed the word "hormone" from a Greek root meaning "to stir up, or excite," and Cunningham uses the term in its original meaning, which is lost sight of in speaking of "endocrines" produced by "glands of internal secretion." Certain substances, ordinarily regarded as waste products of cellular metabolism, are known to affect the activities of organs, and have been called *parahormones.* Thus carbon dioxide activates the respiratory center of the medulla and is administered with oxygen to avoid respiratory collapse; and the normal heart-beat of some lower vertebrates (*e.g.*, sharks) seems to depend upon a certain concentration of urea.

While much is known as to the *effects* produced by hormones, little is known about the *mechanism* whereby these effects are produced. It appears that a probable explanation in many cases may be based on catalyst modification, including inhibitions and the formation of new catalysts. Alteration in membrane permeability is another factor, and from this many catalyst possibilities might later emerge.

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[8] "Heredity and Environment," 1919.

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[11] C. Auerbach, J. M. Robson, and J. G. Carr, *Science* (1947), **105**, 243-247.

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Chapter 9

The Catalyst Entelechy in Differentiation and Morphogenesis

Facts may be recognized and expressed in language, without being understood and explained by having their underlying mechanisms made clear in terms of matter. The ever-wonderful development of a human being from the then unknown fertilized ovum is thus expressed in the 139th Psalm, entitled "God's omnipresence and omniscience":

14. *I will praise Thee; for I am fearfully and wonderfully made; marvelous are Thy works; and that my soul knoweth right well.*

15. *My substance was not hid from Thee, when I was made in secret, and curiously wrought in the lowest parts of the earth.*

16. *Thy eyes did see my substance, yet being unperfect; and in Thy book all my members were written, which in continuance were fashioned, when as yet there was none of them.*

Even after men had freed themselves from kingly and priestly taboos, and began to use their God-given powers of reason and ingenuity, it took centuries of patient study and experimentation by many thousands of devoted seekers after truth to acquire and to learn how to extend our present knowledge of the mechanism whereby we are "made in secret and curiously wrought." Besides the microscope, we now have many instruments such as the ultra-microscope, the electron microscope, spectrographs and spectrometers, with whose aid we can peer into "the lowest depths of the earth" to try to learn what goes on there. And from chemical behavior we deduce the activities of unseen atoms and molecules.

The establishment of important facts, such as the isolation, identification, and often the synthesis of individual chemical substances, as well as the demonstration of the effects they produce, has been the result of much work that fills us with wonder and admiration. But all will end in verbiage unless we carry through and explain how "organizers" organize, how "evocators" evoke, and how "inductors" induct.

The notion that many happenings in morphology and differentiation are understandable on the basis of the direction of chemical change by specific catalysts existing initially in the fertilized egg, or formed or modified in the developing embryo, seems consonant with the experimental data. The formation of various structures—catalysts, cells, organs, tissues—of specific nature and at definite times and places in the developing organism depends upon the formation and assembly of a wide variety of specific chemical molecules at suitable rates, in suitable ratios, and at suitable times and places. Apart from formation *in situ,* the assembly and distribution of small material units is controlled by such factors as Brownian motion, convection, circulatory systems and especially by selective adsorption and differential diffusive mobility. But catalysis is the basic mechanism whereby are produced the particular chemical units which underlie metabolism, development and growth. Catalysis makes these descriptive terms mechanistically and materially intelligible. Without a mechanism they are symbols for unknowns.

When we consider the numerous catalysts and chemical changes dominating what was supposed to be the comparatively simple breakdown of glucose by yeast into alcohol and carbon dioxide, and recall that catalysts may synthesize as well as decompose molecules, we may well stand aghast at the catalyst-based chemical and physico-chemical complexities emerging in a single cell. Furthermore, when we remember that most of the happenings observed and recorded by biologists lie at or above microscopically visible structural levels, it is astonishing to see the wealth of information that has been deduced from critical experiments, which often reach far below what is seen. For example, the presence and location of sulphydryl groups (–SH), which among many other potencies affect the activity of many proteases (catalysts directing protein formation, alteration or destruction), may be made evident by the nitroprusside test. Glutathione, important in growth and containing the –SH group, is thus revealed to be present in the blastoderm* of the chick, but not in the yolk or the white of the unincubated egg.

Since we are here maintaining the view that embryological development and differentiation, in common with other life processes, are directed by what may be called *a material entelechy*

* Blastoderm: The flat sheet formed by the embryo's cell-layers when cleavage is discoidal (Needham).

working largely through the direction of chemical change by catalysis, we must consider what material equipment the fertilized ovum carries with it from its parents. In referring below to what is ordinarily found, we must not forget that in nature curious and unusual things may develop, if they are physically and chemically possible.

This *material* entelechy is something utterly different from the metaphysical entelechy of Driesch, referred to by Professor Edmund W. Sinnott of Yale University.[1] After pointing out that "we have been confusing analysis with solution," and that "it is not an understanding of units we now seek, but of unity," he states that some biologists "have needed little encouragement to run after the strange gods of mysticism and metaphysics and have set up in their midst the golden calf of entelechy. Even those who remain in the ranks of the orthodox speak the unfamiliar language of holism, organicism, metabolic gradients, allometry, organizers, morphogenetic fields, gestalten and other words outlandish in the ear of analytical biology and which for the most part are merely the terminology of enlightened ignorance." Like views are reiterated in his Presidential address before the American Society of Naturalists,[2] in which he states: "Organic form is the external manifestation of this underlying biological organization . . . This integrated and organized behavior, this unity in the midst of diversity, is the most distinctive feature of life in general. What the mechanisms are which underlie it and what is the physicochemical basis by which it is established and persists are deep problems which long have vexed students of development." He concludes: "Let us try, in these days of intense specialization, to cultivate more of the catholicity of the earlier practioners of our science, and especially to implant in our students an interest in and respect for all the branches of biology. From somewhere in this wide field—perhaps from where we least expect it now—may come the next new unifying idea which will show how system relates to substance and how life depends on both."

In the unfertilized ovum, the maternal chromosomes with their genes and aura of adhering substances, are generally afloat in a relatively large pool of maternal cytoplasm. The sperm which enters and fertilizes the ovum brings with it the paternal chromosomes with their genes and adherent material, and a much smaller volume of apparently condensed paternal cytoplasm which

nevertheless is of great importance when the fertilized ovum or zygote develops into an individual—a process dubbed *ontogeny.*

The actual fertilized ovum in the hen's egg, with which we are most familiar, is also very small, the main mass of the egg (its yolk and white) consisting of water and nutrient material sufficient to enable the germ to develop into an individual when the egg is cast off or "laid" by the hen. Such a self-contained egg is termed *cleidoic* (closed box), for it has within its shell everything it needs, except some atmospheric oxygen which can diffuse in, to supplement the air bubble supplied to the egg by the hen. But in some of the lower animals (*e.g.*, sea-urchin, squid, dogfish) the developing embryo absorbs much of what it needs of water, salts, and probably trace elements, from seawater. Mammalian embryos develop a sucker-like attachment (the placenta), and live and grow practically as parasites on the wall of the mother's uterus.

The first mammalian egg observed was that of a dog, by K. E. von Baer in 1827.[3] Mammalian eggs are almost microscopic, ranging from about 70 to 85 microns in rodents (rats, mice, guinea pigs) to about 140 microns in horses, dogs, sheep, goats, pigs, monkeys, apes, man, and whales. Yet this tiny spherical unit, say one-tenth of a millimeter in diameter, contains determinants which enable it regularly to develop, under suitable conditions, into its predestined individual. How is this miracle so regularly accomplished?

As an approach to this problem, let us first estimate what the tiny zygote means in terms of molecules, figuring molecular dimensions to be 0.2 to 5.0 millimicrons, and the zygote as a sphere whose diameter is 100,000 millimicrons. The volume of this sphere ($v=\frac{4}{3}\pi r^3$) is approximately 262×10^9 $m\mu^3$, that is, 262 million million cubic millimicrons. Since a considerable percentage of the molecules are small ones (water, salts) there is ample room, in addition to the chromosomes, enzymes and other inclusions, for many million million highly specific organic molecules supplied by the mother and the father through the ovum and the sperm. Therefore there is plenty of space in the zygote for an enormous number and variety of catalysts, and of specific molecules to serve as modifiers, prosthetic groups and carriers to form, as cell duplication proceeds and as new substances are produced, new catalysts with new chemical ambits.

To envisage what we desire to explain by the catalytic direction of chemical change, consider as one example the following dia-

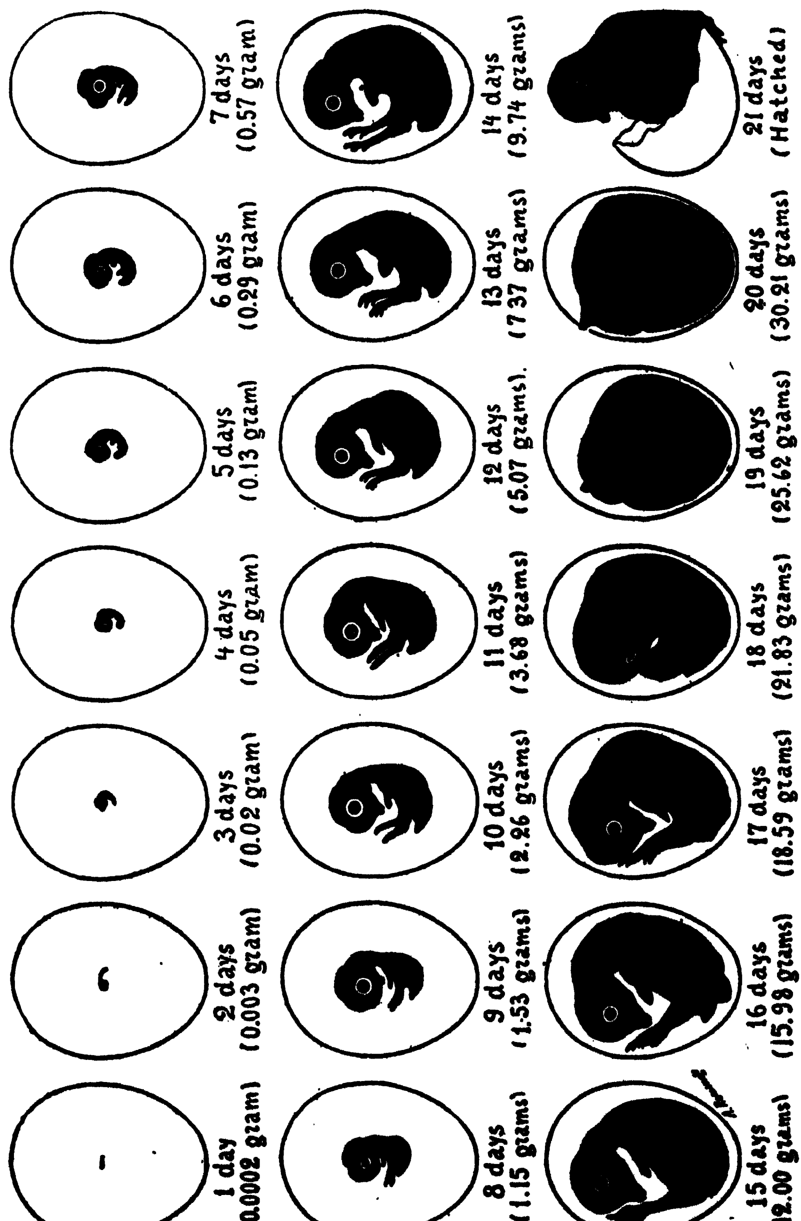


FIGURE 28. *See legend for Figure 29*
(From "Bioenergetics and Growth," by S. Brody, Reinhold Publishing Corp., N. Y.)

grams showing in part what is seen when a zygote develops into an individual. "Everyone has been impressed by the miraculous transformation of the sticky white and yellow mass of hen's egg into a fully dressed, befeathered, respectable chick, all in 21 days.

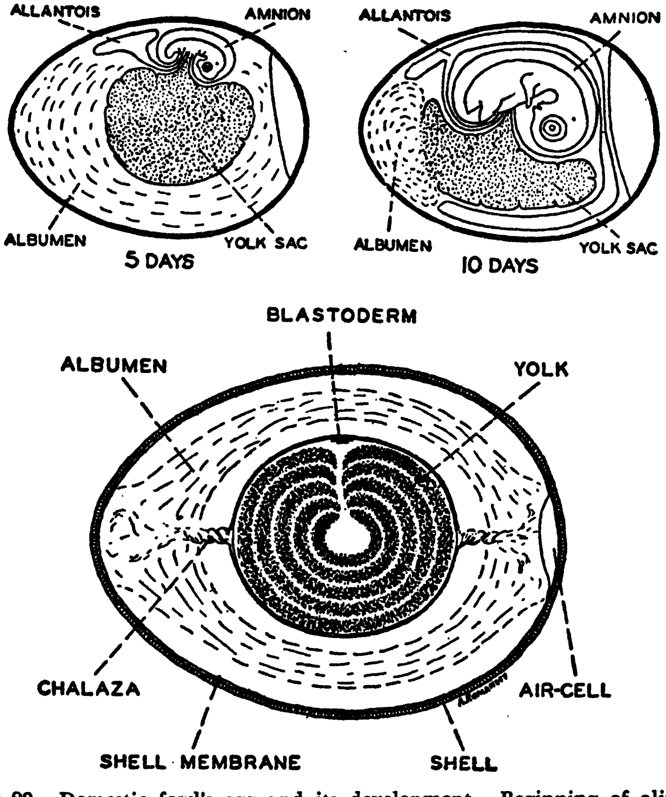


FIGURE 29. Domestic fowl's egg and its development. Beginning of alimentary tract, 18 hours; vertebral column, 20 hours; nervous system, 21 hours; head, 22 hours; blood island-vitellin circulation, 23 hours; eye, 24 hours; ear, 25 hours; heart beat, 42 hours; amnion, 50 hours; legs and wings, 63 hours; allantois, 70 hours; reproductive organs, 5th day; feathers, 8th day; beak turns toward air cells, 17th day; yolk sac begins to enter body cavity, 19th day; yolk sac completely drawn into body cavity, 20th day; hatching of chick, 21st day. *(Courtesy A. L. Romanoff, Cornell Exp. Bull. 205, 1931 and 1936)*. (From Brody's "Bioenergetics and Growth.")

The original egg cell must have travelled at a dizzy pace to build up so complex a mechanism—probably exceeding in complexity* the astronomical wonders with their galaxies and supergalaxies."[4] The following epitome of the development of a mammalian egg is more detailed.

* This, of course, applies to structure levels where stars may be considered as units. On this relatively tiny satellite (the earth) of a medium size star (the sun), untold numbers of similar miraculous transformations proceed daily. J. A.

Though the first few cleavages of the zygote do not greatly increase the size of the cell group, differentiation is already manifest at the 16-cell stage, distinction being evident between the part which later implants the egg on the maternal tissue, and the *trophoblasts,* the cells later to form the fetal membrane, which divide more rapidly and therefore are smaller than the other cells. Next is seen the formation of the *blastocyst,* a vesicle which cups into a hollow sphere that distends with fluids possibly secreted by the trophoblasts; and now the cell mass visibly enlarges.

The outstanding feature of blastocyst development is the absorption of tremendous quantities of water. C. B. Davenport[5] estimated that after six weeks the human egg weighs about a gram, nearly 500,000 times its initial weight; and 98 per cent of the weight increase is water. The blastocyst develops the embryonic envelope and the placenta, the latter really special tissue formed where the circulatory systems of embryo and mother come into contact. The development of the placenta in mammals has been shown by Pincus to be controlled by progesterone, the corpus luteum hormone.[6]

Following attachment of the egg to the placenta, formation of the embryo continues by segmentation and differentiation of the inner cells; and the amnion and yolk-sac vesicles develop and flatten together to form, with an intervening layer of cells (mesoblast), the triple-layered germ disc which comprises the embryo. In man this occurs about the third week, and by the end of the second month the embryo (in the case of man) is recognizable as human. Eyes, mouth region, and limb buds may be seen by the fourth month and the organs are formed the month before. The "ground plan," as Brody puts it, "is laid very early in life and rounded out later through enlargement and remodeling of the parts. Age changes in shape are due to differences in growth rates of the constituent parts. There is an orderly sequence or *gradient*[7] in organ formation. The head has precedence in development over the tail end, and so on in cephalochordal sequence for the other organs. The sequences may be associated with organizer and hormone action."

How is all this orderly development to be explained? The view suggested by W. Roux in 1883 that mitotic division of the zygote establishes development by a qualitative distribution of the nuclear germ plasm was a great advance beyond the medieval notion that a homoculus or "little man" existed initially in the

egg and simply grew larger. Experimental confirmation of Roux' view seemed evident when, in 1888, he reported that if one of the first two blastomeres in the developing frog's egg was killed by a hot needle, the remaining cell developed only a half-embryo.

But in 1891 H. Driesch separated the first two blastomeres of a sea-urchin's egg and was astonished to find that each cell formed a normal though smaller gastrula and larva. The next year he found that even in the four-cell stage, the single cells could produce complete individuals, as could also two eggs fused into each other. It thus became evident that, *initially*, what was to happen to any part of the egg was *undetermined*, even though if undisturbed it had a "prospective significance" (*prospektive Bedeutung*). The fact that any part of the egg could be made to develop quite differently from its normal expectation was due to its multiple potency (*pluripotency*), and the term "prospective potency" (*prospektive Potenz*) covered the total possibilities of the part, irrespective of what might happen to it in any particular experiment.

Driesch was so impressed on finding in the egg this mysterious ability to carry on, for which no material explanation seemed possible, that he invoked a metaphysical vital force or *entelechy*, to account for it, and actually abandoned experimental science to follow philosophical speculation. Notwithstanding this, we believe that the directing vital force is real enough, and that it is explainable on the basis of comparatively simple physicochemical principles, chief of which is catalysis.

Comparative Experimental Embryology

Comparative experimental embryology, as contrasted with observations of naturally developing morphology, received a great stimulus about 1897 when Gustav Born observed the remarkable healing powers of amphibian embryos, and devised experimental techniques which his early death prevented him from utilizing. Fortunately, H. Spemann (Nobel Prize, 1935) made brilliant extensions of this work. With newts it was found that the developmental fates of most embryonic regions are *not* irrevocably determined until *after* a certain stage of gastrulation. Thus if a bit of presumptive tube material from one embryo was transplanted to the presumptive gill region of another embryo, it developed into gills, not neural tube. Presumptive skin, if transplanted into another embryo at a suitable region in what would

become nerve tube, would develop into brain or spinal chord. That is, up to this stage in gastrulation, the regions are undetermined and develop in step with their new actual surroundings (*ortgemäss*), and not according to what their natural undisturbed position would have made of them.

Gastrulation, however, ends this plasticity and irrevocably determines the fate of each part, which can then undergo only its own special type of development, even if grafted on to another embryo. That is, the bit of transplanted tissue now develops according to its *self-contained* possibilities and its *place of origin* (*herkunftsgemäss*), and not according to its new place of growth. Thus an eye region will develop an eye, even though the eye may point inward. As Needham puts it,* the invisible process of determination has ushered in a new period of self-differentiation, in which the embryo has become a mosaic of irreplaceable regions, similar to the development of certain eggs ("mosaic eggs"), which never manifest a period of plasticity or pluripotency.

But one particular region in the amphibian embryo is much less plastic than others—the dorsal lip of the blastopore which has arisen from the grey crescent and will eventually form the mesoderm, notochord, somites, etc. A piece of this region, when grafted into another embryo in the blastula or early gastrula stage, will cause, direct, or *induce* the nearby host tissues to form a secondary embryo, including nerve tube, brain, eyes, ears, somites, notochord, etc., irrespective of what might have developed if no transplantation had been made. So Spemann called this fate-determining portion of the blastopore the *organizer* (in German *organisator*).

Later it was found that local developments in already determined regions, *e.g.*, the induction of a lens from the ectoderm by the eye-cup, were dominated by local inductors. Spemann therefore (1924) termed these *secondary organizers*. Thus, in the case of the eye, if the dorsal ectoderm had not first been stimulated by the "grey crescent" region, no neural tube and therefore no eye-cup would have been formed. So the bit of *living tissue* which heads what Needham terms "the heirachy of organizers in animal development," was called by Spemann the *primary organizer*, while organizers acting at later successive stages are secondary, tertiary, etc. (see Figure 30).

* Needham suggests the following English equivalents: *neighborwise* for ortsgemäss; *selfwise* for herkunftsgemäss.

Then it was found that a dead tissue, a chemical fraction or a substance of known constitution could serve as an inductor; and J. Holtfreter[8] found that certain tissues which when alive did not induce, acquired this power after being boiled. The term *inductor* is therefore much broader than *organizer*, which is limited to living tissue. The chemical substance emitted by an organizer and responsible for all or part of its morphogenetic stimulus was termed by Needham an *evocator*; its action is *evocation*.

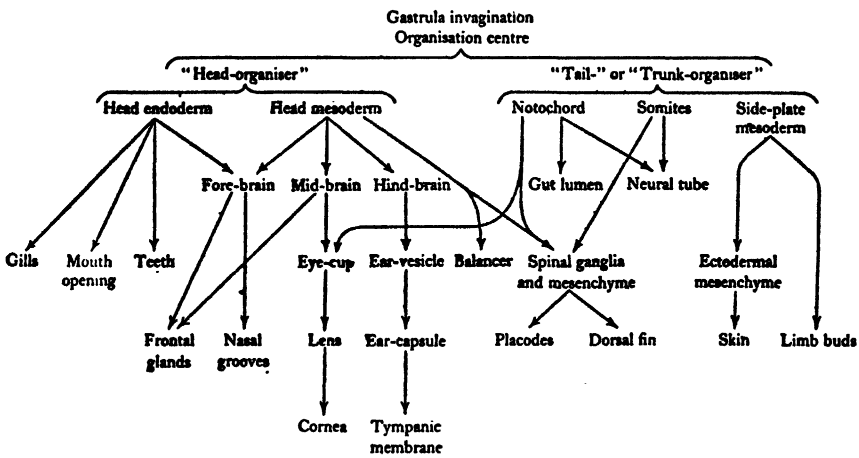


FIGURE 30. The succession of inductors of second and lower grades. (From Needham's "Biochemistry and Morphogenesis," Cambridge University Press, Cambridge, England, 1942.)

But it has been found that stimuli of this kind will be ineffective unless the tissues on which they act are properly receptive. This state of reactivity is known as *competence*.[9] Competence and organizer activity are not retained indefinitely, but may arise and fade away at different times; however the latter appears to begin sooner and to persist longer. Needham states[9a] that no tissue is known which possesses competence but no evocator.

While the terms "competence," "organizer activity," "morphological stimulus" express the facts, they fail to give any inkling of the operative mechanism, which we believe is more nearly approached by the several catalyst mechanisms mentioned below. Thus a tissue may be "competent" to supply a carrier for the prosthetic group of an organizer, or *vice versa*; and these two units may form a specific and active catalyst or enzyme, providing that all conditions (*e.g.*, pH, absence of inhibitors) are favorable. If competence involves, for example, the presence of a labile but powerfully adsorbent protein

carrier, slight change in local conditions may incapacitate the carrier; or adsorption of some diffusate may block it. Then even a steady supply of prosthetic group molecules, from the more stable catalytically functioning organizer, would fail to form the enzyme normally expected. Experimentally, competence would disappear.

By implanting the embryonic dorsal lips of a toad (*Bombinator*) into the blastococle cavity of a newt (*Triton*), Geinitz[10] obtained inductions, thus proving that the primary evocator is not species-specific. Subsequently many other mixed implantations led to successful inductions, *e.g.*, chick in newt, (*Triton*), chick in rabbit, rabbit in chick, zebra fish (*Danio rerio*) in newt (*Triturus torosus*), lamprey in newt (*Molge*). Then Holtfreter[11] made the surprising discovery that the evocator exists not only in the developing embryo, but is also present in all adult tissues of all family groups (phyla). Tests included tissues from two worms, two molluscs, three insects, a crustacean, two fishes, three amphibia, a reptile, two birds, two mammals, and man. Some of these adult tissues manifested inductive capacity without special treatment (*e.g.*, boiling, protein denaturents). Even pieces of the coelenterate *Hydra viridis*, which develops no neural axis, showed a feeble but positive inductive effect on amphibian blastoceles. It has also been reported[12] that implantation of blastomeres from the four-cell stage will induce a neural tube in a competent (or receptive) ectodermal ball.*

Needham believes that the evocator exists in masked or inactivated form in all parts of the embryo except in the organizer region. Masking and inactivation are terms which express results, not mechanisms; but they may be the consequences of the adsorption, or the aggregation, of specific substances which would thereby become incapable of diffusion, until they were eluted, or deflocculated as explained below. Such specific substances might then exhibit electronic fields having catalytic power, or they could then serve as units needed to complete such fields. Adsorption or chemical fixation of specific units may also result in inhibition, masking or inactivation of the adsorbent or the fixing units.

O. Mangold and H. Spemann[13] found that in the course of the determination and histological differentiation of the neural tube, it develops the power of inducing another neural plate when implanted into the blastocele cavity of another embryo (termed *homoiogenetic induction*). As Needham[9a] states: "Its cells acquire induction power, and in later development the evocator appears

* A dispersion of 7-day chick embryo extract in Tyrode solution produces inductions most nearly approaching those of a living amphibian organizer. To Dr. Lillian Baker of Carrel's laboratory fell the main task of keeping alive the bit of chicken heart in a continually renewed chicken embryo juice medium.

free in all tissues." This is readily understandable on the basis of the catalyst mechanisms discussed below. Needham also points out many resemblances in behavior between the primary organizer and the plant hormones, auxin and "bios," which also can exist in "bound" condition, show no species-specificity, and are "unmasked" subtly. He also remarks that the retort of Prof. W. Lash Miller to critics of Miller's and other early work on "bios", "is not without moral for embryologists." Miller said: "All those who say, 'No doubt some chemical in the wort makes all the difference, but it need not be Bios', have qualified to join the Last Ditch Bacon Club, which holds that Shakespeare's plays were written, not by Shakespeare, but by some other person bearing the same name."

The Chemical Nature of the Primary Organizer

By use of various solvents and precipitants, fractions acting as organizers have been prepared; but as their inductive capacities vary with the treatments, it may be that the organizer consists of more than one substance or else becomes altered or broken down in the separation process. It seems to be of steroid nature, a view supported by the work of Waddington, Needham and co-workers.[14]

L. G. Barth[15] obtained excellent inductions of brain tissue by implantation of cephalin prepared from mammalian brain. Then Fischer and collaborators[16] obtained inductions of neural tubes by implantation of thymonucleic acid and of adenylic acid derived from muscle. Later they found both purified and synthetic oleic acids to be effective, as well as linolenic and octadecenic acids and nucleo-protein from calf thymus; so they believed that acid stimulation is responsible, a concept which the buffer action of cell groups (tissues) renders most indefinite.

Methylene blue in high dilution causes induction.[17] Thus a piece of ventral ectoderm isolated from the gastrula and cultured two days in $M\ 10^{-5}$ methylene blue nutrient induced a large brain in a host, and being competent, became neuralized itself. A similar isolate became neuralized by longer cultivation in methylene blue medium. Histochemical tests revealed in the gastrula ectoderm (the presumptive mesoderm and presumptive neural plate) a substance termed "plasmogen," later found to be a phosphatide in which aldehydes of higher fatty acids are combined with glycerol, phosphoric acid and amino-ethylalcohol; it is related to the

important cytoplasmic nucleotides and shows the Feulgen reaction.

Woerdeman[18] observed a sharp disappearance of glycogen from the dorsal lip during invagination. Now it had been shown by Willstätter and Rhodewald[19] that two forms of glycogen exist in the cell, one readily soluble (lyoglycogen) the other comparatively insoluble (desmoglycogen); and it was found that both of these could induce neural tubes, because they contain, in adsorbed state, some ether-soluble material in amounts so small that after 50 hours' extraction in a Soxhlet apparatus the solution, on evaporation, left a scarcely visible residue. Yet this trace material was a potent inductor.

The view here being maintained is that the mechanism whereby an undifferentiated (neighborwise) group of cells becomes converted, irreversibly as a rule, into a differentiated (self-wise) group, is a *transmissible, i.e., cellularly heritable, change in biocatalysts.* This leads to a change in chemical output having physiological and morphological consequences. Similar, but progressively more specific and limited catalyst changes determine or are associated with the formation, activity and location of secondary, tertiary and subsequent organizers, until the various structural fields exhaust the existing possibilities and enter the adult stage of equilibrium. That the equilibrium is a dynamic one, at least in some respects, is shown, *e.g.*, by the growth of skin, hair and nails, the healing of wounds, and the development of abnormalities such as tumors and acromegaly.

How do there arise in a cell new or changed catalysts which are transmissible to a daughter cell upon cell division?

Nature will, no doubt, utilize somewhere every possible effective mechanism, and no limitations on nature are suggested in the following discussion of what appear to be the principal mechanisms:

(1) Gene mutation
(2) Gene modification
(3) New catalyst formation
(4) Deflocculation and elution

Before considering these possibilities further, it should be pointed out that cellularly heritable change seems to be a basic factor in the following:

(A) The "*Anlagen.*" An *Anlage* is a cell group which develops into a certain organ, *e.g.*, liver, heart. Though not always dis-

tinguishable in early stages, these cells tend to breed true in tissue culture.

(B) *Morphogenetic fields or gradients.* The field is an ordered pattern of instabilities set in a cellular matrix.[20] The field effect results from an equilibrium position, and the term *gradient* stresses the increase or decrease of field potency from one point or pole to another.

(C) *Individuation,* the field forces which render asymmetrical the neural axis (arising from the chemical stimulation of the evocator) by differentiating head end and tail end.

Needham[9a] says that "individuation is one of the most difficult problems confronting embryology today . . . the possibility is by no means excluded that other chemical substances may be at work in the formation of the normal neural axis besides the primary evocator. The existence of second grade inductor substances is, of course, beyond doubt. . . . But we may also find it worth while to distinguish between substances which stimulate tissues to form an organ (inductors) and substances which modify in various ways the shape of organs so induced. If such substances exist, it is at any rate clear that the position in which they are normally liberated is so exactly controlled by the individuation field of the inducing tissues that the regional correspondence of inducer and induced is secured. Waddington[21] has discussed the possible existence of these substances or *Eidogens* in a short review. The important thing which head-organizer does as opposed to tail-organizer is to modify the cross section of the neural tube to form a brain rather than spinal chord. Inductors and eidogens would thus be the mechanism by which the individuation field of the inducer controls that of the induced."

This last statement follows the very common but erroneous notion that the demonstration of chemical compounds, and even their isolation, complete analysis and synthesis (though all this is of primary and vital importance) make clear the *mechanism* by which they act. For example, the "bios" problem was under attack for many years before Professor F. Kögl (Utrecht) in 1936 isolated biotin, which noticeably affects the growth of yeast in dilutions of one part in 400 billion. Its structure was unravelled by K. Folkers and V. du Vigneaud,[22] and biotin itself was successfully synthesized in the laboratories of Merck & Co.[23] But this admirable work of itself does not demonstrate the mechanism by which biotin acts. It seems, however, that individua-

tion and other Gordian knots of embryology may be cut by the versatile sword of a physico-chemically dominated catalyst entelechy.

We now consider the four classes of transmissible catalyst changes before mentioned.

(1) Gene Mutation

Professor Richard Goldschmidt devotes a large portion of his book "Physiological Genetics" to "The Mutated Gene and the Potentialities of Development." At the outset he states:

"One of the basic facts of genetics is, then, that the action of the gene in controlling the development of hereditary traits cannot be studied directly but may only be extrapolated from the knowledge of the action of a mutant gene: the existence of the normal or +-gene is only inferred from the existence of a mutant allelomorph showing Mendelian behavior. The action of the mutant gene is a different one from the assumed action of the +-gene; *i.e.,* the mutant gene must control or produce a deviation in a series of developmental processes leading to the visible character. Development . . . is . . . a process of extraordinary precision . . . the possibilities of changing the details of developmental processes without injuring the proper cooperation of those processes are rather limited. . . . But certain processes may be changed without deleterious consequences; and if this is done by a genetic change, we call it a mutation."

Professor Theodosius Dobzhansky[24] states: "For the time being the term *mutation* subsumes a variety of phenomena. In a wide sense, any change in the genotype which is not due to recombination of Mendelian factors is called a mutation. In the narrower sense, it is a presumed change in a single gene, a Mendelian variant which is not known to represent a chromosomal aberration."

From our point of view a gene mutation is an intraparticulate change (chemical or physical) which results in outwardly directed changes in the gene's electronic fields of force, sufficient to alter its catalytic activities. Conceivably, this could be brought about by intramolecular change, by a change in molecular orientation, or by electroversion.[25] Where a genic unit (molecule, macromolecule, or aggregate) changes its contacts, as in cases of chromosomal translocations, inversion, deletion, or crossing-over, a new catalytically effective surface may be formed at the new zone of contact with other genes or with the milieu, as the case may be. The so-called "position effect" in genetics can be thus accounted

for. It is a question of language as to whether this is to be called a mutation—in physical effect it may be.[69]

As far back as 1903, H. Devaux[26] found that, when a lens of molten fatty acid (stearic) was allowed to chill on a water surface and was then carefully dried, the top surface of the lens which had set in contact with air was water-repellant (hydrophobe) because of the outwardly directed hydrocarbon groups; whereas the bottom of the lens could be wet, because there the water-loving (hydrophile) COOH groups were outwardly directed. Work on surface phenomena by Sir W. B. Hardy, W. D. Harkins, I. Langmuir, N. K. Adam, The Svedberg and many others indicates how potent may be slight changes in pH, trace substances, etc., in affecting the nature of a surface, even though nothing or the merest trace be added directly to it.

The publication (1927) by H. J. Muller[27] of work showing that both gene mutations and chromosomal changes can be brought about by x-ray and other short-wave irradiation* exerted a profound effect on genetics and particularly on ideas regarding mutation. While mutations produced by x-rays appear to be like those which occur spontaneously, the mutation rate is greatly increased—about 200 times in some cases. Many species of plants (from fungi to flowering plants) and animals (from protozoans to mammals) have been heritably changed, and recently a filterable virus. Cosmic, solar, and telluric (via uranium or thorium) radiations are possible factors demanding further investigation.

In a recent review,[28] Muller states: (1) "As to the mechanism of production of those radiation mutations which affect individual genes or narrowly circumscribed chromosome regions, the most important fact that has come to light is the dependence of a given mutation upon a change that was initiated in a single individual atom, by its ionization or other excitation." (2) Structural changes occur in the chromosomes because of breaks in the chromatin and a two-by-two fusion of the ends derived from different breaks.

Genes at the new junctions or new free ends of chromosomes often show "position effects" probably due, as stated above, to the formation of new or modified catalyst fields.

In his argument that the individual genes as separate units do not exist, R. Goldschmidt[29] states that "many effects that originally were regarded as due to gene mutation have turned out to be position effects of rearrangements." Assuming "that the whole chromosome is a large chain molecule of complex arrangement,"

* Professor Muller (Indiana Univ., Bloomington) received the Nobel prize in 1946.

he states: "All points taken together suggest strongly that the chromosome is the actual heredity unit controlling the development of the Wild type, that purely steric changes[30] at the individual points of its length produce deviations from the Wild type which may be described as mutations, even as point mutations, though no actual Wild-type allelomorph and therefore no gene exists." "If the chain is intact, and each residue is in its proper steric position, the catalytic processes dependent upon this chain molecule occur in a way that leads to what is called the *Wild type*. Any change in the chain, however, of whatever type, may disturb the interplay of the catalyzed reactions, and a deviation occurs which is called a mutation." The final words of his book are: "It remains for the physico-chemist to decide whether or not the new model could also take care of the independent catalytic actions of what was considered to be a gene."

The structural and mechanically operative nature of the chromosome is not made clear, nor is it fairly expressed by the words "a large chain molecule of complex arrangement." Work with the various textile fibers[31] indicates that in them there are many structural levels above the macromolecular chains, and that these chains form bundles which in turn are bound into still larger micells, probably in part by the cohesive action of adhering substances and by interpenetrating fibrils. Whatever the structure of the chromosome, it seems to be more complex than a simple protein chain molecule of the nature described by M. Bergmann, even though many such molecules may be in the chromosome. Goldschmidt's abandonment of the gene is rather verbal than factual, for even if considered as a side-chain in a macromolecule, it is still there as a specific atomic complex. His views have been adequately critized by Dobzhansky.[32]

It is, however, encouraging to see that so eminent a biologist as Goldschmidt appreciates the biological importance of catalysis, even though his language seems to limit it to the gene. In dealing with embryological questions he fails to invoke catalysis. His quandary regarding the position effect of rearrangements is readily clarified by the fact that the genes, as well as many other biological units such as the enzymes, exert their specific catalytic effects because of their outwardly directed electronic fields, and the question of importance is the nature and directive power of these fields rather than the terms we use to describe the units which establish the fields. Thus three units CAT would exhibit dif-

ferent electronic fields if rearranged into ACT; and this would be true whether the letters represented atoms, radicals, molecules, macromolecules, or even larger units.

(2) Gene Modification

If a gene group adsorbs a particulate unit, such as an atom, ion, or molecule, and the gene is able to duplicate itself so as to maintain the new specific catalyst surface consequent upon the adsorption, we have the same effect as a gene mutation. Something allied to this occurs when a carcinogen like 3:4-benzpyrene acts to make cells heritably cancerous, for the carcinogen itself is not reproduced, though the cell culture continues cancerous.

Even if a gene that has been modified by adsorption were to duplicate itself in *unchanged* form, the new catalyst area could be maintained in the cell clone* providing:

(a) the new catalyst surface could direct the formation of particles of such specificity that, when adsorbed by the *unmodified* gene surfaces appearing on gene duplication, the modified gene surface (with the modified electronic field) would be formed.

(b) the food, milieu, or the activities of other catalysts furnished a continuous supply of the specific modifying particles.

The ability to adsorb specific particles previously not adsorbable might be one of the consequences of a gene mutation; the specific effect would not be produced in the absence of the specific adsorbable particle.

(3) New Catalyst Formation

The attachment of a prosthetic group to a suitable carrier may form a specific catalyst surface, which may serve as a mold or template from which are produced more areas *of like electronic contour*, to serve in turn as prosthetic groups for additional catalyst areas having a specificity like that of the first-formed catalyst unit.

We pointed out in the chapter on immunology what happens when a piece of tin-foil is pressed against a newly-minted coin: the surface of the foil which lies *next* to the coin acquires the *reverse* impression of the coin surface, but the top surface of the foil forms a *duplicate* impression of the coin surface. In like manner the thin molecular "plaque" formed against a new catalyst

* A *clone* (also spelled *clon*) is a group of cells having a common ancestor, which they generally resemble. The term is derived from the Gaelic *klonn*, a twig, and is allied to the Scotch word *clan*.

area has essentially the *reverse* electronic impression on its near side, but a *duplicate impression on its off side.* If on leaving the molding surface the plaque is adsorbed by some other surface (which may be a particulate carrier) in such manner that its off side faces the milieu, we may have a workable duplication of the original catalyst area. (Adsorption the other way round may lead to antibody formation).

This notion of new self-duplicating catalysts does not gainsay the dominating importance of the chromosomes and genes, but it does indicate how even *one single molecule* could serve to establish the catalytic formation of new substances in a cell clone. In fact it has been reported[33] that one crocin molecule is able to induce a sexual change in an alga. The position of the gene is sufficiently secure to permit even the most devoted followers of T. H. Morgan to admit the existence of non-genic heredity of the catalyst type; or they may prefer to consider a new autocatalytic catalyst as a symbiont or an extrachromosomal gene.

Let us now consider some of the experimental evidence indicating that cells are able to form new and heritable catalysts.

Apart from the many specific catalysts known to exist in yeasts, it has long been known that yeast can be "trained" to ferment certain sugars. Nearly half a century ago F. Dienert[34] found that a yeast which was unable to ferment galactose acquired this power when small but continually increasing percentages of this sugar were added to the fermentation mixture.

Karström[35] divided the enzymes of microorganisms into two groups: (1) *constitutive enzymes,* whose formation is common to all media; (2) *adaptive enzymes,* which are formed only if the cell is "stimulated by the presence of a specific chemical substance in the milieu," a procedure which recalls the formation of antibodies in animals as a response to an antigen. On experimenting with nine strains of yeast (*Saccharomyces*), H. E. Rhoades[36] found that each strain could ferment glucose, mannose, sucrose and raffinose regardless of the carbon source in which the cells had grown; that is, these sugars were fermented by constituent enzymes. On the other hand, maltose, trehalose, alphamethylglucoside and galactose* were fermented only if the organism had been "trained" by growth in the presence of the specific substrate or a closely related substance, that is, after the formation of adaptive enzymes.

Evidently, yeast can "manufacture" new enzymes to meet new

* One strain (S. cerevisiae Hansen) possessed the inherent ability to ferment galactose.

situations, and it appears that the new substances which the yeast "learns" to ferment add their own specificity to a system of molecules to make a new prosthetic group. (See also the work of T. M. Sonneborn in Chapter 8.)

(4) Deflocculation and Elution

These may liberate carriers and/or prosthetic groups, which can then become effective as gene modifiers or as catalyst formers; or pre-formed enzymes may be freed from inhibitors or from an inactivating adsorption.

The Svedberg (Nobel prize, 1927)[37] and his collaborators found that the molecules of the respiratory protein hemocyanin from the snail *Helix pomatia* shows, when slightly acid (pH 6·8), a molecular weight of 6,740,000; but when made slightly alkaline (pH 8·0) the protein splits into three components whose molecular weights are 6,740,000, 3,370,000, and 842,000 respectively. Restoration of the pH to 6·8 causes the fragments to reunite. Dissociation of proteins may follow high dilution, or the addition of an amino acid or another protein. As little as 0.001 per cent of thyroxin causes an appreciable dissociation of thyroglobulin. The action of a dissociating compound on a protein is more or less specific. Thus in the presence of ammonium chloride, arginine dissociates serum albumin but not *Helix* hemocyanin, while lysine plus ammonium chloride splits the latter protein but not the former. Guadinine chloride affects *Helix* hemocyanin very strongly, but serum albumin only slightly. Clupein splits both proteins, but arginine, in the absence of ammonium chloride, affects neither.

Epitomizing the work on the important respiratory proteins, which are interesting from a taxonomic as well as a physico-chemical point of view, Svedberg distinguishes two groups:

(1) Respiratory proteins active in cells: *e.g.*, Keilin's cytochrome-C, Warburg's "yellow enzyme" (now known to be of complex nature), and myoglobin ("muscle hemoglobin") which takes up and gives off oxygen along a dissociation curve, and seems to serve as an oxygen reservoir for the organism. The first two take part in cellular oxidation-reduction reactions.

(2) Respiratory blood proteins, falling into the following classes:
- (a) red pigments (erythrocruorins, hemoglobins)
- (b) green pigments (chlorocruorins)
- (c) blue pigments (hemocyanins)
- (d) reddish-brown (hemerythrins)

Classes (a) and (b) have similar iron-containing prosthetic groups, and can take up one molecule of oxygen for each iron atom. In class (c) the prosthetic group contains copper, and these proteins can take up one molecule of oxygen to two atoms of copper. In class (d) the prosthetic group also contains iron, but the oxygen-binding capacity is one oxygen molecule to three atoms of iron. The cellular respiratory proteins cytochrome-C and myoglobin both have iron in their prosthetic group and have about the same molecular weight (17,000) one-fourth that of hemoglobin (68,000) which has four iron atoms per molecule, and is found in the blood of all vertebrates except the lowest class, the *Cyclostomata*. Mammalian hemoglobin dissociates reversibly upon addition of certain amino compounds, *e.g.*, urea, acetamide, formamide.

"The hemocyanins form an interesting class with a number of inner connections. The molecular weights of the hemocyanin molecules found in the blood of certain species are always simple multiples of the lowest well defined component. Thus, for the *Malacostraca* the relationship is 1:2 and for the *Gastropoda* 2:8:16:24. Moreover, the weights of all the well-defined hemocyanin molecules seem to be simple multiples of the lowest among them. In most cases the hemocyanin components of certain species are interconnected by reversible pH-influenced dissociation-aggregation reactions. At certain pH values a profound change in the number and percentage of the components takes place. The shift in pH necessary to bring about reaction is not more than a few tenths of a unit. Consequently, the forces holding dissociable parts of the molecule together must be very feeble.

"Not only the molecular weights of the hemocyanins but also the mass of most protein molecules—even those belonging to chemically different substances—show a similar relationship. This remarkable regularity points to a common plan for building up protein molecules. Certain amino acids may be exchanged for others, and this may cause slight deviations from the rule of simple multiples; but on the whole, only a very limited number of masses seems to be possible. Probably the protein molecule is built up by successive aggregations of definite units, but only a few aggregates are stable. The higher the molecular weight the fewer are the possibilities of stable aggregation. The steps between the existing molecules, therefore, become larger and larger as the weight increases."

All this indicates how delicate is the physico-chemical mechanism controlling the particle size of proteins, which may affect catalyst formation, and thereby determine the pH and the formation or deflocculative liberation of specific substances that may exert profound effects on protein structure and function, *e.g.*, as

carriers and prosthetic groups for still other catalysts. Changes in aggregation and particle size will also greatly influence kinetic activity, diffusibility, passage through septa, adsorbability, and the nature and extent of the outwardly directed electronic mosaic. With the biologically important colloid proteins, as indeed with most other substances, we must, at a certain stage of organization, envisage the possibility of particulate unions and dissociations which are not comprehensible in terms of simple stoichiometry, even though they are due to residues of the same subatomic potencies which are responsible for chemical attraction. There comes a zone in the successive levels of increasing complexity of material structure, where the externally directed electronic mosaics of the particulate units may be somewhat variable and affected by adsorbed "impurities," and yet the attraction (or repulsion) of the units may be biologically important (*e.g.*, consider mitosis and "crossing over"), although not as powerful or as precise as with true chemical combinations between smaller and less organized particulate units.

As to *elution*, we need only recall the masterly work of Richard Willstätter,[38] who concentrated enzymes several thousand fold by adsorbing them on suitable adsorbents, filtering off the exhausted fluid, and then stripping off or *eluting* the adsorbed enzyme from the adsorbent by slight changes in the pH or the chemical nature of the dispersion medium. Besides being important in many analytical procedures (*e.g.*, chromatography), this process of selective adsorption and differential elution has extensive industrial applications; for example, the important drug penicillin was concentrated from highly dilute dispersions by adsorption on activated carbon, and was eluted from the carbon by a suitable solvent.[39]

An eluate may, in turn, serve as an elutor of other substances from the adsorbed state; or it may act as a prosthetic group, a carrier, a complete enzyme, or as an inhibitor of enzymes.

It must also be considered that the formation and release, at catalyst surfaces, of substances of low diffusive mobility may establish local concentrations of these substances far higher than their average distribution as shown by an overall chemical determination. In fact, a number of geneticists have applied this notion of accumulation to explain the "position effect."[40] Increased length of the diffusion path tends to limit the distribution of the output of a catalyst surface, and this, in turn, may affect

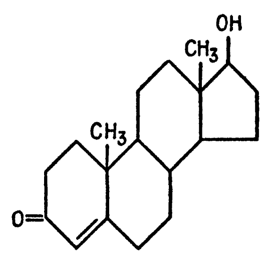
the "raw material" supply and therefore the output of other catalysts.

Selective adsorption, followed by elution, could also establish local concentrations sufficiently large to permit a substance to function in any of the just-mentioned capacities of prosthetic group, carrier, catalyst, elutor, or inhibitor. The work of Svedberg and his school, above mentioned, adumbrates how profound may be the consequences for catalyst formation or modification. On the other hand substances locally formed may be carried elsewhere to exert specific effects—the hormones, for example.

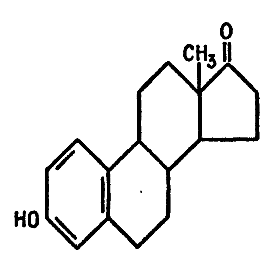
The interplay of these numerous possibilities indicates that in these simple mechanisms the cell has ample scope for the most diverse activities. The potency of local accumulations of trace substances might well be called "trigger action."* The formation of new carriers and prosthetic groups makes possible new enzymic catalysts, which, in turn may direct the formation of still other chemical outputs and structures as the material entelechy develops.

Sex Determination

Very slight chemical differences may lead to enormous physiological and morphological differences at higher structural levels. Consider the close resemblance of the chief mammalian male and female sex hormones which exert such potent but characteristically different influences. For example, when twin calf embryos are



Testosterone
(male sex hormone)



Estrone (*or Theelin*)
(female sex hormone)

genetically of different sex and the union of the fetal membranes establishes continuous circulation, the sex hormone of the developing bull calf causes the female calf to develop into a *freemartin*, a sterile intersex with testes and male ducts.[41] Following

* Iodide therapy may precipitate an attack of acute lead poisoning, by converting lead phosphate stored in the bones into soluble lead iodide.

injections of female hormones, roosters become "broody" and will hatch out and care for chicks; while under the influence of male hormones, hens become combative and will "tread" other hens. Injections of male hormones make non-singing female canaries assume male courtship behavior and sing like males. Chick embryos of both sexes develop combs and spurs, but those of the female soon degenerate. If the left (functional) ovary is removed from a very young female fowl, the bird assumes male characteristics, its right ovary becoming functionally a testicle; and it is reported that a normal fertile hen has been changed to a "normal" fertile rooster.[42]

Although Prof. C. E. McClung[43] discovered the sex chromosomes about half a century ago, it is only recently that their operative mechanism has been made evident. The expression "genic balance" applies to sex characteristics and indicates that many genes play a rôle.

The action of sex-controlling genes, presumably through sex substances formed by their activities, may be glimpsed from the following resumé of the development of the sexual system in amphibians, based mainly on Needham's description.

What are to become the germ cells arise from the dorsal wall of the gut endoderm. They become embedded in the wall of the splanchic mesoderm as it moves in to form a median dorsal mesentary plate. This plate splits lengthwise into two bundles which move outwards. Next, chains of cells from the blastema of the mesonephros migrate into the gonad and form a medulla or central core. The endodermal germ-cells are thus connected with two sorts of mesoderm: a cortex having female potentialities, and a medulla having male potentialities; and no other mesoderm has like competence. It had been shown by M. Laulanié[44] that in the case of a genetic female, the cortex develops at the expense of the medulla and induces the formation of eggs; whereas in a genetic male, the medulla develops at the expense of the cortex, and induces the formation of sperms. Though egg cells and spermocytes both have the unique power of reduction division which halves the chromosome number,* the two haploid sperms survive, one with an *x* chromosome, the other with a *y* chromosome; but only one of the haploid egg cells (with an *x* chromosome) survives, the other being aborted and cast out as a "polar body." This recalls, though at much different structural levels, the freemartin effect above mentioned, and also the instinct which leads a queen bee to sting to death all other queens in her domain.

* The subsequent longitudinal splitting of the chromosomes in "equatorial division" does not affect the haploid number.

Considering now the amphibian sex duct systems, the Wolffian duct, with male potentialities, arises as the pronephric or segmental duct on each side of the body. The Mullerian duct, with female potentialities, generally originates independently from the epithelium of the coelom. These two form all the later ducts, and both of them appear in every organism; but the particular sex hormone poured into the blood stream determines which of the two systems will persist and develop. The non-developing system normally degenerates into a vestigial structure; but we can understand how in abnormal cases there may develop intersexes or hermaphrodites. The various secondary sex characters (external genitalia, accessory sex glands; wattles, spurs and combs of fowl) are all controlled by the sex hormones.

Sex reversal is known to occur spontaneously (Pflügerian hermaphroditism), and as before noted can be artificially induced. Oysters are usually male when young, but become female as they grow older. The Florida silver palm also changes sex.[45] With the sex substances there seems to be a balance, controlled in part by temperature and differential diffusibility. If two frog larvae of opposite sexes are grafted side by side and develop toward maturity, the male medulla will suppress the cortex of only the nearer ovary. But if the larvae are grafted in tandem, no effect is noticed, the diffusion path being too long. With urodeles (newts) side-by-side grafting suppresses both ovaries of the female partner. The effect may be quantitative; for if the female of a large species is grafted to the male of a small species, the ovaries suppress the testes. High temperature and over-ripeness of eggs favor maleness, *e.g.*, the medulla over the cortex. By grafting an ovary into a cool spot (ear or scrotum), involuted sex organs of castrated mice have been restored to normal, apparently by the small amount of androgen supplied by the ovary; but grafting onto the warmer abdomen was without effect.[46]

By injections of oil solutions of estrone into the allantoic cavity of birds' eggs on the fourth day of incubation, V. Danschakova and J. Massavičius[47] completely feminized the embryos of genetic male birds. But the introduction of testosterone proprionate to effect masculinization was lethal in birds, though with guinea-pigs the freemartin effect was produced, the male hormone suppressing feminization.

The case of the gephyrean worm *Bonellia* is fantastic. From the body of the female, about the size of a walnut, there projects a yard-long probosis which serves to collect the nutrient-containing mud. The tiny male, less than 3 millimeters in length, lives as a commensal in the female's intestine and uterus. Less than one percent of the larvae spontaneously develop into males. Ninety-nine percent of the free-swimming larvae will become females unless they happen to

become fastened by a sticky secretion of certain larval glands, to the probosis of some female worm, where their gradual development into males can be followed. Those who have experimented with this rather rare worm, speak of the number of "probosis hours" in the life of such a male.

Artificial Parthenogenesis

Recent research in artificial parthenogenesis has developed facts of great interest here. E. B. Harvey[48] obtained by ultracentrifugation nuclei-free fragments of sea-urchin eggs which, when activated by hypertonic seawater[49] in many cases gave rise to a single cytaster (and frequently to several cytasters). The cytaster was followed by an amphiaster and division of the cell between the two asters. Afterward, when cell division does not carry through, the cytasters multiply within the unsegmented fragment. Sometimes the merogens develop as far as the 500-cell stage and form free-swimming blastulae, before development ceases. The egg cytoplasm evidently carries sufficient directive agents for the early stages of development, but chromatin or nuclear material appears necessary for gastrulation and further differentiation. J. Needham comments that the failure of these parthogenetic merogens to show the Feulgen reaction (used to identify nuclear material) is inconclusive, for though there must be much of this ribo-nucleotide type nuclear material in the cytoplasm anyway, it cannot bring about gastrulation; neither has the external addition of nucleotides, vitamins or hormones.

Waiving the question as to the possible presence of dispersed nuclear material in the non-nucleated merogen, A. Tyler[50] believes that in normal mitoses asters arise in connection with, and are probably due to, the activity of a self-perpetuating central body, even though Harvey[51] did not describe definite central bodies in the cytasters. For as far back as 1901 E. B. Wilson,[52] figured them quite clearly in his cytological observations on activated non-nucleated fragments; and more recently Fry[53] showed that differences in cytological technique may greatly affect the appearance of the central body. Therefore Tyler believes "it is reasonable to assume that the appropriate method would demonstrate distinct central bodies in Harvey's material too. It appears then that the production of a cytaster involves in effect the *de novo* formation of a self-multiplicative, genetically continuous body."

Some Instances of Inductor Action

The importance of nuclear inductors in determining developmental form or morphology is brought out by the striking work of J. Hämmerling[54] with the umbrella alga, *Acetabularia,* whose huge single cell may grow to 2 or 3 cm. high, the rhizoid or root-like base having a long stem terminating in an "umbrella" 0.6 cm. in diameter. The umbrella is preceded by a series of wreaths of hairs, and consists of a number of radial chambers which later contain the sex cells (gametes). The single nucleus is in the rhizoid end, away from the umbrella, but from this nucleus there diffuse substances which can specifically direct regeneration of either end of the plant. Thus if the plant is cut in two, the nucleated rhizoid end will reproduce an umbrella. The other end, having no nucleus, may live quite a while, but before eventually perishing it may regenerate an umbrella or a rhizoid. The regeneration of the umbrella takes place more readily the nearer the cut is to the "umbrella" end, while the opposite is the case with the regeneration of the rhizoid. But in the absence of the nucleus the amount of regeneration is sharply limited.

Hämmerling concluded that the nucleus produces two directive substances which diffuse differentially so that more of the umbrella-forming stuff accumulates toward the umbrella end, and more of the rhizoid-forming stuff remains nearer the supplying nucleus. When a nucleated piece was allowed to begin regeneration and the nucleus was then removed, regeneration proceeded even if the cut exposed part of the stem which would not ordinarily form an umbrella; for the nucleus had supplied the necessary form-directing materials. Their stability is indicated by the fact that if the umbrella is cut off two months after removal of the nucleus, a new umbrella is regenerated. If the stem is cut near the root, an umbrella will develop at this unusual place; but if the nucleus is removed before completion, regeneration will cease after the exhaustion of the umbrella-producing substance.

Are genes within the nucleus involved in the formation of these directive substances? An affirmative answer was given by further experiments with two species of the alga whose umbrellas showed differences in color, in number and shape of their chambers, etc., namely *Acetabularia mediterranea* and *A. wettsteinii.* On grafting anucleate fragments of one species onto nucleated fragments of the other, in both directions, it was found that the nature of the nucleus determined what kind of umbrella would develop. Thus a *mediterranea* stem grafted onto a nucleated *wettsteinii* rhizoid, gave a typical *wettsteinii* umbrella. Goldschmidt remarks: "This shows that actually the genes within the nucleus control the production of a specific formative stuff (not

unspecific, as in the hormonic type) which diffuses through the cytoplasm to the place of its form-controlling action." Owing to the complex nature of the resulting umbrella, the formative material may consist of several substances; or a substance with several catalytic potencies; or else it must initiate in the cytoplasm of both species the same "entelechy" of catalyst formation and chemical change.

After describing Hämmerling's work, Needham states: "From all that has been said in the preceding sections, it will have become clear that the genes act in development by producing, inhibiting the production of, or masking and unmasking, hormones, catalysts or inhibitors in more or less diffusible states. Hence their undoubted effects upon rates of reaction in the embryo. *It might even be said that the main difference between genetics and embryology is that in the former study only those inductors are described which are unable to pass through the cell-membranes of the cells in which they are formed, while in the latter, only inductors of a more diffusible nature are described.* But this would leave out the whole question of competence, which is likely to remain mysterious long after our present knowledge of genes and inductors has quadrupled its present state."

It is strange that Needham should see such mystery in competence, which he defines as "the state of reactivity of a part of an embryo, enabling it to react to a given morphogenetic stimulus by determination and differentiation in a given direction." As pointed out above, the formation or activation of specific catalysts will commonly call upon local tissues to bear some part of the burden, *e.g.*, by supplying carriers, prosthetic groups, elutors, or suitable pH; and if the prosthetic group should find the essential carrier already preempted by some other group, the prospective catalyst may not be formed. By blocking one reaction in a catalytic chain, the absence of a single catalyst may alter the local chemical output, and therefore the formation of substances and structures which are criteria of physiology as well as of determination and differentiation.

Some Abormalities

Let us now consider some of the consequences which may follow if the usual course of catalytically directed chemical change is upset. The normal catalyst entelechy is all askew.

First come the naturally occurring abnormalities termed *teratomata,* defined as a group of histologically differentiated tissues (often

malignant) embedded in an otherwise normally developed organism, and showing various degrees of morphological differentiation. Despite the chaotic assembly of various tissues and organs (in one case dozens of tiny "tonsils" in separate little patches of "pharynx"), the specific glands may function and the muscle be capable of contraction. By injection of 5 per cent zinc chloride into the testis of a fowl, H. J. Bagg[55] found in many the development of "teratomata" sometimes big enough

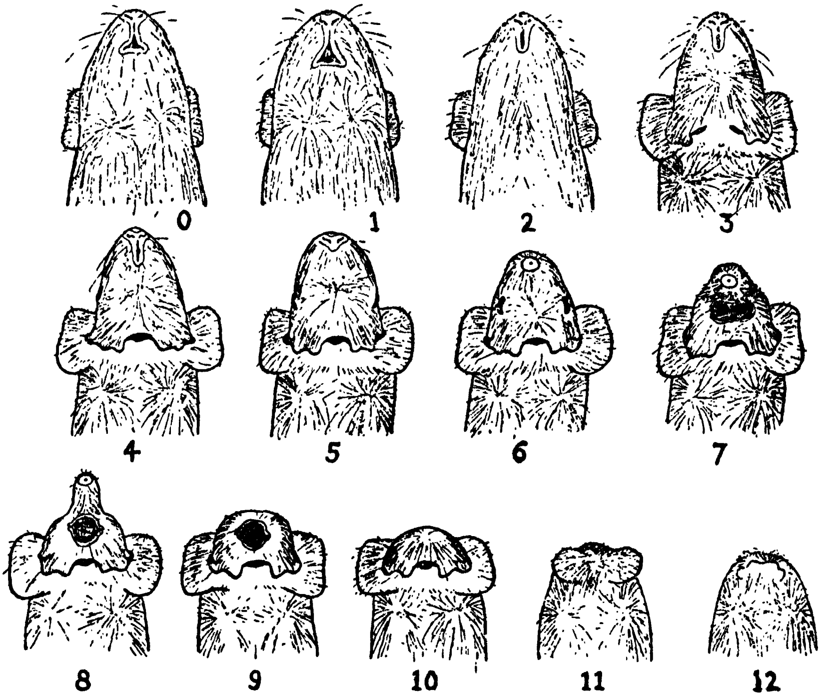


FIGURE 31. Grades of otocephaly. Semi-diagrammatic ventral views of the head and throat of the 12 grades in comparison with the normal (o). [Courtesy *Journal of Agricultural Research*, Vol. **XXVI,** No. 4 (1923)].

to fill most of the visceral cavity, and containing cartilage, bone, fat, muscle, skin, feather follicles, glands, and also nerve, connective and cancerous tissue. Since zinc chloride is a powerful disperser of some proteins (it will fluidify glue or gelatin), it might well be that a variety of specific proteins (prosthetic groups or carriers) in the fowl's gonads or sperms are dispersed and set free to act with the carriers or prosthetic groups normally present in adult tissue, but functionless unless converted into active catalysts by appropriate combination.

Other groups of abnormalities, mainly involving malformations, where the animal or plant departs greatly in form or structure from what is usual for the species, are termed *monsters.*

They may, for example, lack certain organs or parts or have too many limbs. I have seen a bull with two heads, one quite small and imperfect. A well-known instance is the heritable abnormality otocephaly (loss of head) in guinea-pigs found by Sewall Wright,[56] which probably results from inbreeding. While the rest of the body is normal, the following graded degrees of interference with head formation are found, which F. E. Lehmann[57] believes to be due to a defect in the head organizer: in Figure 31 (1) lower jaw is reduced; (2) no mandible palpable externally; (3) ears connected under throat by bare skin; (4) single median ear opening on throat; (5) mouth and upper incisors lost; (6) nostrils fuse; (7) eyes are in contact below a narrow nasal probosis or are more or less fused; (8) eye fusion complete; (9) probosis lost; (10) eye lost; (11) ear opening lost; (12) body rounds off in front of shoulders, with no sign of a head except a small single median external ear.[58]

Goldschmidt states that the various defects which combine to make otocephaly are traceable to a small number of centers of abnormality: ventral mandibular arch, olfactory placodes, cerebral vesicles, median optic rudiment and some others.

Embryological investigation of the heritable x-ray induced mutations produced in mice by C. C. Little and H. J. Bagg[59] made by K. Bonnevie[60] and G. M. Plagens[61] showed that while the abnormalities may be much diversified, *e.g.*, lack of eye (anophthalmia), extra digits (polydactyly), club foot, etc., they follow as a consequence of an overflow of medullary fluid in the neck of the embryos when they are 7 or 8 milimeters in length. The blebs or blisters thus produced move about uncertainly as embryonal growth continues, and interfere with normal development. What the recessive mutant gene does is simply to establish the conditions leading to the release of the medullary fluid; and since this happens at an early stage, quite a variety of final effects may follow.

Similarly, it is uncertain what may be the consequences of an embolism in the circulatory system of a human being. It might kill by obstructing the circulation in the heart, the brain, the lungs, etc. Hence the wisdom of absolute rest in phlebitis, so that no dangerous blood-clot is set free while redispersion of the large local clot is proceeding.

We now mention a few of the many cases where developmental abnormalities have been experimentally produced by the presence or the lack of definite chemical substances.

F. E. Lehmann[62] found that trichlorbutyl alcohol, a narcotic which does not inhibit the primary evocator in the dorsal lip of the amphibian blastophore,[63] can inhibit the secondary evocators which direct the formation of a lens by an eye-cup. The degree of inhibition, up to complete absence of lens, depends upon the temperature (less at 10° than at 22°), on the narcotic concentration, and also on the stage of development when the narcotic is applied. After the lens-forming ectodermal thickening had appeared, lens formation proceeded despite the narcotic, which seems incapable of interfering with the catalyst system, once this is established. Before this, the narcotic may possibly block off some carrier or prosthetic group.

Needham points out that "These facts raise the question of the naturally-occurring blindness in cave-dwelling animals. There can be little doubt that it involves a series of effects due to interference with the inductor chain brain→eye-cup→lens→cornea at various different points."

An eye-cup brought experimentally into contact with undetermined ectoderm for 24 hours induces lens formation; and ectoderm, transplanted from a distant region to a place over the eye-cup, developed a lens. But Spemann found that in many amphibia, lens formation can take place without eye-cup assistance; in fact, in the frog *Rana fusca* and the newt *Triton vittatus* lens-forming competence is distributed all over the ectoderm. In the toad *Bombinator,* any part of the head ectoderm will form a lens, but trunk ectoderm will not. In the frog *Rana esculenta,* only the presumptive lens district showed competence (ability to form a lens following stimulation by the evocator).

These facts show the danger of framing a theory on the basis of too limited a group of experiments, even within a very restricted field. An acceptable theory of life processes must be broad and versatile enough to be consistent with all experiments in any field, no matter how remote from the field in focus. The catalyst theory of life seems to meet this criterion, despite our present inability to unravel the enormous intricacies in a single cell.

Returning now to the cave-dwelling animals, they and probably also their foods, are protected from direct solar radiation, though possibly more exposed to such telluric radioactive radiations as might exist in their habitat. Apart from any effects which might be produced by abnormal or mutant genes, it is well known that eye-cup and other suppressions such as loss of hind limbs may be produced in pigs by restricting the mother's dietary

vitamin A, a substance related chemically to carotene and other lipochromes (retinene, astacin) which are functional in the eye region. Thus the Texas State Agricultural Experimental Station reported in 1932 that a Duroc-Jersey gilt on a vitamin A-deficient diet farrowed eleven pigs all without eye-balls. F. Hale repeated the experiment,[64] and one pig was born without any eye-cup at all.

It is interesting to note that vitamin A (carotene) serves as a prosthetic group in the conjugated protein system serving vision in the higher animals. The equilibrium visual purple (rhodopsin) $\rightleftharpoons$ visual yellow (retinene) $\rightleftharpoons$ visual white concerns the retinal rods, while the visual violet (iodopsin) is involved in the retinal cones useful in night vision and especially important to nocturnal animals, *e.g.*, owls. To prevent "night blindness" aviators wear special red glasses before flying at night, in order to protect their visual cones and render their night vision acute.

Among cave-dwellers some of the eye deficiencies reported are the following:

In the salamander *Proteus anguineus* cornea induction is inhibited by a thick wad of connective-tissue mesoderm which develops between the eye-cup and the epidermis. In the blind cave-fish *Anoptichthys jordani* lens induction spontaneously fails. The lizards *Amphisbaena* and *Rhineura* lack lens and cornea.

The blind fishes and blind amphibia form series showing various graded degrees of eye abnormalities, from degeneration of the retina following normal development, to absence of lens, of cornea and of eye-cup differentiation. The caves also have many blind invertebrates, *e.g.*, the shrimp-like crustacean *Niphargus.* Reference should here be made to the work of Prof. George H. Parker on neurohumors, and of Prof. Francis B. Sumner on the effects of visual stimuli on the colors of fishes and amphibia.

These facts raise the question as to what extent deficiencies in foods and in solar radiation (due *e.g.*, to long periods of intense volcanic activity, as in the upper Cretaceous), may result in abnormalities under natural conditions. Thus J. Alexander stated:[65] "The riddles presented by the disappearance of various animal species and human tribes are, of course, open to many possible interpretations—change of climate, pestilence, extermination or submergence by other animals or tribes. A more subtle but equally potent possibility is food failure—not necessarily actual famine, but a deficiency in the supply of certain foods

which furnish small quantities of essential substances, or the failure of the plants or animals furnishing such substances to produce them in sufficient quantity."

V. L. Colucci[66] and G. Wolff[67] independently reported that, on removal of the lens from a larval or an adult amphibian, a new lens develops from the upper margin of the iris. Recent experiments with this "Wolffian regeneration" showed that if small bits of iris are snipped off and pushed into the eye-chamber (*corpus vitreum*), lenses develop there; but bits of ectoderm acted likewise. With *Rana esculenta* Wolffian regeneration fails in the presence of a normal lens, but if the lens is removed, lenses form on implantation into the eye of neural plate tissue, ectoderm, eye-cup, somite mesoderm and ear vesicle. Tissue cultures of chick iris are reported to have produced lenses. It might well be that the eye-chamber can supply specific prosthetic material, while the other diverse tissues supply the specific carrier material, or *vice versa.*

The secondary organizers responsible for lens formation are stable enough to stand boiling, and like primary organizer they seem to be present in all tissues. But the amount is limited locally, for on repeated extirpation and regeneration the third lens in the series was poorly developed, and no fourth lens appeared. Prolonged rest restored the regenerative power; either the active substance had been reformed locally, or else the widely distributed organizer had diffused in. If the latter possibility is true, it would be evidence in favor of the view advanced by J. Alexander[68] as to how the gametes (ovum and sperm) receive the enormous number and variety of particulate units which are needed to form, or to serve as templates for forming the specific catalysts, prosthetic groups or carriers needed to carry out the material catalyst entelechy. Alexander stated: "How can heritable factors, apart from genic or chromosomal changes, enter into and affect the cells which carry heredity?

"It is reasonable to believe that the cytoplasm of germ cells, like that of other cells, tends to receive an average supply of the molecular flotsam and jetsam, that is, of all particulate units which are carried throughout the body by circulation and diffusion, and that among these molecular or near molecular units are those that determine or aid in determining, differentiation. Apart from other evidence, the fact that some differentiated cells can breed true in tissue culture warrants the belief that specific

tissues or organs contribute to the molecular melée in the organism units capable of determining their own specific type of cell."

The simple processes of circulation and diffusion may thus help to lift from the genes and the chromosomes some of the tremendous responsibilities placed upon them by considering them as the *sole* carriers of heredity. The great numbers and varieties of specific molecules trailing along in the gamete cytoplasms, and especially numerous in the ovum, act through the catalyst entelechy as cooperators with and supplementors of the genes.

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[14] They obtained inductions with the following polyhydrocarbons and steroids (structural formulas given by Needham, *lib. cit.*, pp. 245-6):

Estrogens: 1:9-dimethylphenanthracene
9:10-dihydroxy-9:10-di-*n*-butyl-9:10-dihydro-1:2:5:6-dibenzanthracene
estrone (female sex hormone)
4:4′-dihydroxy-diphenyl
1:2 dihydroxy-1:2-di-α-naphthyl-acetnaphthene
5:6-*cyclo*-penteno-1:2 benzanthracene

Carcinogens: 3:4-benzpyrene
methylcholanthrene
styryl blue
sodium 1:2:5:6-dibenzanthracene-endo-α-β-succinate*

* Optimum dosage (yielding 40 per cent inductions where implant was retained) was 0.0125 γ per embryo. (S. C. Shen, *J. Exp. Biol.* (1939) **16**, 143).

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[29] *lib. cit.*, p. 309ff.

[30] According to L. Zechmeister [*Chemical Reviews* (1944) **34**, 278] the calculated number of stereoisomers for naturally occurring carotenoids are, for example, as follows: α-carotene, 32; β-carotene 20; γ-carotene 64; lycopene, 72; crocetin, 20; vitamin A, 4. (J. A.)

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In 1907 J. H. McClendon [*Biol. Bull.* (1907) **12**, 142; *Arch. Entw. mech.* (*Roux*) (1908) **26**, 662] had sucked out the nucleus from an echinoderm egg and fertilized parthogenetically the non-nucleated cell, or "merogen," that remained.

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[58] Fig. 27 and these descriptions are taken from the original paper by Sewall Wright and Orson N. Eaton, *J. Agr. Research* (1923) **26**, 161-181. H. B. Adelmann [*J. Exptl. Zool.* (1929) **54**, 249, 291, and subsequent volumes to 1937; *Quart. Rev. Biol.* (1936) **11**, 161, 284] found that in normal development the two eye-districts overlap, but the intermediate section does not develop into eyes because the prechordal plate inhibits it. If this inhibition is obstructed, either mechanically or by the action of specific substances, *e.g.*, lithium, a single eye (cyclopia) results. C. R. Stockard (1913) produced cyclopia in a sea urchin (*Fundulus*) by treatment of the early embryonic stages with magnesium salts. In the heritable eye-abnormality termed *coloboma*, there is incomplete coalescence of the eye bulb. By feeding turpentine to a pregnant rabbit a similar malformation (non-heritable) has been produced in the young. The hatteria (*Sphenodon punctata*), a lizard-like reptile survival of ancient times found only in islands near New Zealand, has a pineal or median "eye," corresponding to vestiges found in snakes. This eye seems to have no visual function, though it was more fully developed in various extinct groups of reptiles and *Stegocephali* (H. F. Gadow, Cambridge). Possibly it is sensitive to slight differences in temperature.

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[62] *Arch. Entw. Mech.* (1934) **131**, 333.

[63] A. Marx, *Arch. Entw. Mech.* (1931) **123**, 333.

[64] *J. Heredity* (1933) **24**, 105.

[65] "Some Physicochemical Aspects of Life, Food and Evolution," *Scientia*, October, 1933.

[66] Mem. d. R. Accad. dell'Inst. di Bologna, 1891.

[67] *Arch. Entw. Mech.* (1894) **1**, 380.

[68] "Colloid Chemistry," Vol. V, p. 596, Reinhold Publishing Corp., 1944.

[69] J. W. Mellor (Vol. V, p. 720, [1924]), quotes Lucretius (60 B.C.) as follows: "It matters much with what others and in what positions, the same atoms are held together. . . . When the configuration of the atoms is changed, the properties of the body which is formed from them must also change."

See also the fourth line in the quotation from Lowell on page 248.

Chapter 10

Some Catalytic Aspects of Disease and Drugs

Disease is defined as "any departure from, failure in, or perversion of normal physiological action in the material constitution or functional integrity of the living organism." This indicates that an understanding of diseases must be based on a consideration of what happens to the biocatalysts, whose activities are responsible for material structure and physiological action. Biocatalysts determine what substances will be formed in the organism, where and at what rates they appear, how they will be chemically combined or altered, and whether they will persist unchanged. Abnormalities in the biocatalysts or their activities often are the basic causes of disease; but every abnormality does not necessarily result in disease. For the most part diseases have been classified on the basis of clinical "syndromes" (concurrent aggregate of symptoms) having constant pathologic conditions, and for convenience have been grouped according to the part of the body most affected—heart, lungs, brain, stomach, eyes, nose and throat. Progress in scientific medicine has been largely due to our ability to trace disease processes to lower and lower structural levels and to discover how happenings at these lower levels affect the clinical picture which confronts the practising physician.

Knowledge that many diseases follow infection by microorganisms is so widespread that it is difficult for us to hark back to the days when Anton von Leewenhoek (1632-1723) first saw "little animals" with his home-made microscopes and astonished the Royal Society with his findings; when Francesco Redi (1626-1695) and Lazaro Spallanzani (1729-1799) disproved "spontaneous generation"; and when Louis Pasteur (1822-1895) read his epoch-making papers "Lactic Acid Fermentation" and "Alcoholic Fermentation" (1857). But we are all familiar with pasteurization of milk, beer and other foods, with the Pasteur preventive treatment against rabies, and with the happy results so generally gained from antiseptic surgery and treatment of wounds introduced by John Lister

(1827-1912), which paved the way to modern aseptic surgery. Yet in 1863 Pasteur told the Emperor of France that it was his ambition "to arrive at the knowledge of the causes of putrid and contagious diseases." This recalls the remarkable statement of Robert Boyle (1627-1691) that whoever could discover the nature of ferments and fermentation would be more capable than any other of explaining the nature of certain diseases.

In 1876 Robert Koch (1843-1910), who later discovered the bacteria of tuberculosis, cholera, and other diseases, confirmed the earlier observation (1863) of Devaine that minute rods (bacteridia) exist in the blood of animals dead of anthrax, and are the cause of the disease. Pasteur confirmed Koch's work and convinced those who opposed it. Finally in the spring of 1881 in the farmyard of Pouilly le Fort at Melun (France) came dramatic evidence of the value of immunization: twenty-five sheep protected against anthrax by Pasteur's vaccine survived massive inoculation with these virulent germs, while every one of a like number of unprotected sheep died of anthrax. Protection of human beings and of animals against infectious diseases of many kinds is now extensively practised, and we have developed an ever-increasing number of substances which will kill or inactivate pathogenic microorganisms without too much harm to the patient.

The struggle to understand the basic causes of diseases has led, in many cases, from the infecting organisms themselves to the injurious substances which they produce (exotoxins, *e.g.*, diphtheria) and/or of which they consist (endotoxins, *e.g.*, tuberculosis). Our knowledge of the mechanism whereby definite chemical substances produce definite clinical syndromes is only slowly emerging; but we are here aiming to demonstrate that the lowest efficient structural level where the damage becomes determinative of disease is that of biocatalyst structure and activity. "Poisons" may act (1) by massive chemical attack (strong acids or alkalies), which crudely destroy both the catalysts and their protecting milieu; (2) by disintegration of catalyst structures, *e.g.*, by separating carriers and prosthetic groups, or by "lysis" of essential catalyst structures (snake venoms); (3) by coagulation of biocatalysts or of their milieu; (4) by inhibiting the activities of essential biocatalysts (cyanides); and (5) by establishment of new or modified self-reproducing biocatalysts under whose influence damage is done to the organism, as in cancer, to be considered below.

It must be stressed, however, that such catalyst formations or

changes do not necessarily produce disease; their effects may be unimportant or even beneficial. They may be basic in evolution, for example, by leading to the production or modification of structures, odors, etc. more attractive to the opposite sex, by giving superior resistance to predators or microorganisms—in short by establishing an effective superiority over competitors. Furthermore, every new self-duplicating catalyst may not be persistent. It may be inactivated or destroyed by the immunological mechanism of the cell; indeed, recovery from disease is often effected in this manner.

In final analysis, cellular death comes when the stoppage of the circulation of the blood deprives the cellular biocatalysts of their necessary sources of supply and elimination, and they are soon disintegrated or irreversibly coagulated by the changed milieu conditions. Brain cells are particularly sensitive to lack of oxygen (anoxemia), which can quickly produce irreversible damage to them. The cellular chromosomes speedily clump after somatic death (the death of the organism as a whole): Professor H. A. Evans (University of California) and his collaborators had to work with great speed to demonstrate the 48 separate chromosomes in human somatic cell.[1]

Nature and Classification of Diseases

Some years ago[2] the writer attempted to outline a classification of diseases dependent rather upon physicochemical principles than upon the part of the body most noticeably affected, and often carrying the name of the discoverer (Bright's disease, Paget's disease). A revised form of this large table is herewith presented, for it offers a "bird's-eye" view of what goes on in the human body, and stresses the necessity of having breadth as well as depth of mental focus in considering so highly complicated an organism. Naturally there is no attempt at completeness in a diagram of this kind, but it will serve to outline many of the main interrelations between structure, function, health, ageing, disease, and death. Certain details for which there is no space in the diagram are represented there by numbers which refer to accompanying notes where some details are given.

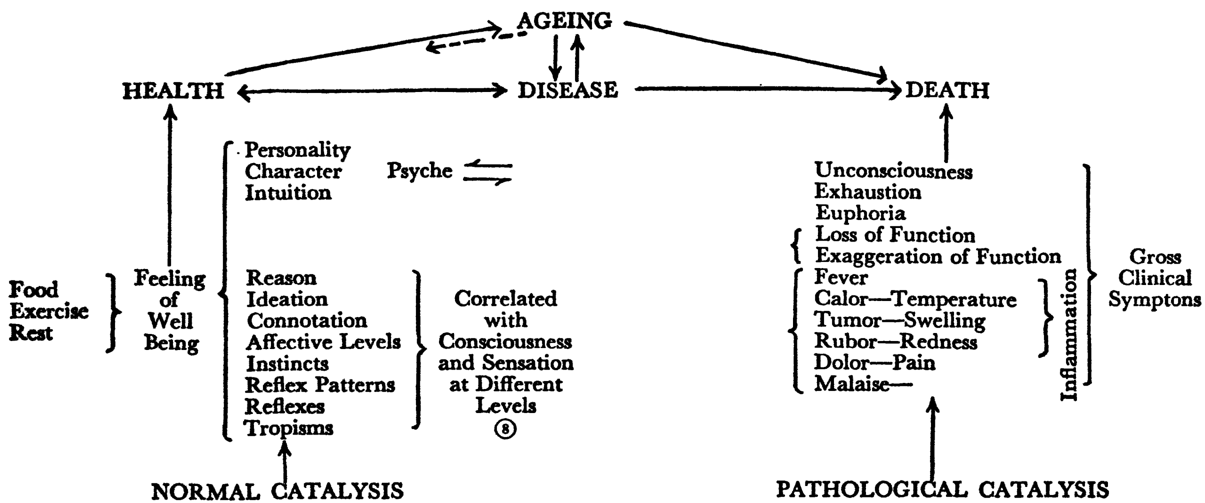
Discoveries in various sciences have enabled medicine to peer more and more profoundly into happenings at progressively lower structural levels of the organism, and thus gain a deeper knowledge of the basic causes of various diseases. As the science of

DISEASES

Structural and Functional		⇄	Infectious
Hormonal	—Diabetes mellitis Acromegaly Exopthalmic goiter		*Ultrafilterable Viruses* Rabies, Influenza, Smallpox Poliomyelitis, Yellow fever
Enzymic	—Apepsia		*Bacteria* Endotoxins—(typhoid, tuberculosis) Exotoxins—(diphtheria, tetanus) Mechanical block—(anthrax?)
Traumatic	—Acute and occult		
Emboli	—Cassion disease (bends)		
Psychic	—Nervous and mental		*Protozoa* Syphilis, malaria, Amebic dysentery
Allergies	—Hay fevei Some asthmas		
Ageing	—Senility		*Fungi* Actinomycosis Athlete's foot, ringworm
Poisoning	—Gases (CO, H_2S) Pb, As, Se, *etc.* Alkaloids, *etc.* Ptomaines		*Vermes* Tape, pin, hook worms Liver-fluke
Excesses	—Alcohol, drugs		*Insects* Bott or warble fly
Dietary	—Avitaminoses Inanition Lack of trace substances		
Immunological	—Inability to produce antibodies		
Neo-catalytic	—temporary or transmissible changes in biocatalysts (cancer)		

medicine thus increasingly added to the clinical picture, it went far beyond Galen's recognition of the following "temperaments": (1) sanguine; (2) phlegmatic; (3) bilious or choleric; (4) melancholic. Later, medical men spoke of *diathesis,* a predisposition to certain forms of disease. For example, *hemorrhagic diathesis* was defined as "a morbid condition marked by lessened coagulability of the blood and an abnormal liability to bleed at slight wounds"; and the Greek term *hemophilia* did not add to knowledge of the cause, even though the condition was observed to "run in families", *e.g.,* the Spanish royal family.

Individuals differ widely in their ability to form and maintain antibodies capable of protecting them against common infections and antigens. The rather unusual disease termed *noma,* a gangrenous condition of the mouth and some other orifices, seems to be due to inability of the individual to form the usual protective antibodies. Hutt states[3] that resistance to disease usually depends on more than one gene. But in one recorded case a simple recessive mutation was responsible for the loss of an entire strain of animals; this happened in guinea pigs that were unable to form blood complement.[4]



The combined activities of the various systems outlined on page 220, are directed and integrated by catalysts, with the outcomes indicated above (see Note 10, page 221)

(Continued next page)

STRUCTURAL AND FUNCTIONAL SYSTEMS OF THE HUMAN BODY

Order of Size	Level of Size	Support	Motion Oxidation	Control (Sensing) (1) Nervous System	(2) Chemical Hormones	MAINTENANCE AND GROWTH (8) — CIRCULATION REPAIR — SUPPLY			ELIMINATION				Reproduction	Protection	Stabilizations
Visible Particles over 50μ	Visible (Anatomy)	Bones Cartilages Bone Marrow	Muscles Tendons	Brain Plexes Nerves		(3) Lungs (Oxygen) (4) Digestive Tract (Food) Stomach	Lymphatics Lymph	Heart Arteries Veins Capillaries	Liver Gall Bladder Bile Gall	Kidneys Bladder Urine	Lungs Carbon Dioxide (CO_2)	Skin Digestive Tract	Gonads Sex Organs	Reticulo-Endothelial System Spleen Anti-Bodies Agglutinins Opsonins Complement (Immunity) Detoxification Phagocytes Spleen Liver	H-ion Concentration or Reaction (pH) Nerves Hormones Circulation Lungs Water Liver Kidneys Skin Tissue to Tissue Transfer Nervous System Temperature Hypothalamus Skin Nerves Capillaries Evaporation Sweat (Salt) Oxidation—Reduction Potential (rH) Immunity Active Passive Mineral Salt Balance
Microscopically Resolvable Particles over 100 mμ	Microscopic (Histology)	(5) Tissues	Tissues	Tissues		Tissues	Tissues	Tissues	Tissues	Tissues		Tissues	Tissues		
		(6) Cells Calcium Deposits Chromosomes	Cells Striated Smooth	Cells Neurones		Cells	Cells	Cells Leucocytes Erythrocytes Platelets — Rouget Cells Controlling Capillaries	Cells	Cells Glomeruli Tubules	Cells Muscle Epithelial Ciliated	Cells Seat Glands	Cells Ova Sperm		
		GENES, ENZYMES, PROSTHETIC GROUPS, CARRIERS, MODIFIERS											DIFFERENTIAL DIFFUSION		
Colloidal Particles 2–100mμ	Ultra-Microscopic (Colloid Chemistry)	Genes Enzymes Sols-Gels	SURFACE SPECIFICITIES (7) GLYCOPROTEINS			Food Dispersed into Colloid Sols, Emulsions or Simple Molecules which can Diffuse through the Gut	SELECTIVE ADSORPTIONS PROTEINS			FATS					
Molecules 0.2–5mμ	Molecular (Chemistry)	Proteins Lipins Water Salts Carbohydrates etc.													
Atoms 0.1–0.6mμ	Atomic (X-Ray Spectrometry) (Physics)		ANALYSES ↓ AND SYNTHESES OF NUMEROUS ↓ CHEMICAL INDIVIDUA ↓ LS — CARBOHYDRATES				AMINO ACIDS			LIPIDS					
Atomic Nuclei	Nuclear (Physics)		RADIOACTIVE AND OTHER ISOTOPIC TRACERS												
			OXIDATION—REDUCTION POTENTIAL												
Electrons	Electronic (Physics)		INVOLVED IN CHEMICAL CHANGES AND IN NERVE CONDUCTION												

(See p. 221 for numbered footnotes)

① NERVOUS SYSTEM	② HORMONES	③ AIR	④ FOOD	⑤ TISSUES	⑥ TYPICAL CELL	⑦ ENZYMES	⑧ SENSES	⑨ FORMATIVE PERIOD	⑩ SUMMATION	⑪ EVOLUTION OF LIVING UNITS
Automatic Sympathetic Parasympathetic Thoracico-Lumbar Bulbo-Sacral Cerebro-Spinal Motor Sensory Integrative Nuclear Cortical	See Table of Hormones	Quantity Quality Purity Sufficient to Supply Adequate Oxygen Evaporation at Skin (Cooling Effect) ——— Absence of Excess.... CO_2–CO (A certain amount of CO_2 is essential to stimulate the respiratory center, etc.)	Quantity, Quality Variety Sufficient to Supply Adequate Water Salts NaCl, Fl, I Fats Carbohydrate Proteins } Calories (Must include all essential Amino-Acids) Roughage Vitamins, etc. See Table, of Vitamins	Specialization ORGANS Specialization	Cell Wall or Interface Cytoplasm Nucleus Chromosomes Chromomeres Genes Genels Golgi-System Mitochondria ——— Adaptations Due to Special Positions	Salivary Ptyalin Stomach Pepsin Pancreas Trypsin Diastase Steapsin Liver Lipsae Diastase Gut Entero-Kinase Steapsin ——— Respiration Ferment in Man = Fe Compounds Horse Shoe Crab = Cu Compound Phallusia = V Compound (With Free Sulphuric Acid in the Blood)	Sight Hearing Smell Touch Taste Vestibular (Balance) Special Sense Receptors Pain Temperature Vibration Protopathic Epicritic	Function Influences Structure ——— ADULT PERIOD Structure Influences Function "As the twig is bent so is the tree inclined"	The Summation of the Structural and Functional Systems (indicated by the Greek Σ) Integrates (indicated by the Integration sign ∫) into Health through Normal Physiology: into Disease or Death through Abnormal or Pathological Physiology (*See bottom of p. 219*)	Moleculobiont First Life (Hypothetical) Genes Filtrable Viruses Gene Strings or Groups Bacteria (Bacteriophage) Mildews Molds, etc. Monocellular Plants and Animals Multicellular Plants and Animals

One good feature of these older clinical aspects is that they stressed the fact that patients differ widely in potentialities, and that it is not alone a stereotyped "disease" which must be treated, but also the variable reactions of patients having the disease, or exposed to conditions which produce it. These mainly genetic differences in individuals in colonies of animals kept for experimental purposes, and even in litter mates, were discussed at a Conference on Animal Colony Maintenance,[5] and constitute a criterion in judging the results reported on animal experiments. In his introductory remarks Dr. Edmond J. Farris (Wistar Institute) stated that in his undergraduate days "type" experiments in mammalian physiology "hardly ever agreed with the textbook picture. This was common classroom experience."

Dr. F. B. Hutt of Cornell University points out that the physiologist, the pathologist, and particularly the nutritionist want a steady supply of animals showing minimum variability. "The geneticist, on the other hand, thrives on variability. It is his stock in trade. The recalcitrant rat that lingers on long after its orthodox litter-mates have terminated their abbreviated careers on the diet lacking vitamin Q is merely a statistical nuisance to the nutritionist, but to the geneticist it is the potential progenitor of a race able to manage nicely with much less vitamin Q than millions of other rats that are less fortunate in the matter of genes . . . Because some biologists still think that genes cause only such 'sports' as freaks of hair color, of hair form, of eye-color, or other inconsequential mutations, but have little or no effect on fundamental physiology, these examples are chosen to refute that view."

In yellow mice the gene A^y (one of a series of allels affecting hair color in the common house mouse) is lethal to the homozygote, which dies *in utero*. The mice born alive show a ratio of 1 non-yellow to 2 yellow heterozygotes, which latter are characterized by (1) adiposity; (2) a subnormal metabolism; (3) slightly greater body size, apart from their excessive fat; (4) lower susceptibility to spontaneous mammary carcinoma than their black or brown litter mates.[6] In fowl a dominant autosomal gene produces the condition "frizzle," the feather curling back toward the head. When "frizzles" are mated, the progeny show three types: so extremely frizzled as to appear woolly; standard frizzled type; not frizzled. The ratio is 1:2:1. The extremely frizzled birds become more or less bare as their feathers break off; and these homozygous birds differ from normal fowl in (1)viability; (2) rate of growth; (3) age of sex maturity; (4) metabolism; (5) rate of heart

beat and size of heart; (6) size of endocrine glands, etc.[7] A type of dwarfism in the house mouse is caused by a simple recessive mutation which, while not affecting secretion of the gonadotropic hormone by the anterior pituitary, interferes with secretion of its growth-promoting hormone. H. Grüneberg[8] lists the following effects of this mutation: (1) endocrine malfunction; (2) adult weight about one-quarter of normal; (3) complete sterility in both sexes; (4) histological abnormalities in interior lobe of the pituitary; (5) small, abnormal thyroids; (6) subnormal metabolism; (7) infantile structure of thymus, adrenals and gonads; (8) subnormal viability.

Enough has been said to show that there is much yet to be learned about the real underlying causes of predisposition to disease (diathesis). Uric acid diathesis was defined as "a condition of the system in which uric acid is deposited in the joints." In Sir William Osler's great book[9] the following appears in the discussion of gout:

"Uric acid, in the body almost completely in the form of urates, is the end-product of purine metabolism in man, just as urea is the end-product of nitrogenous substances of amino-acid and pyrimidine origin. In man the end product of purine breakdown is excreted as urate, there being no enzyme in man to break it down further to allantoin, as occurs in most mammals." Curiously enough, Dalmatian coach dogs differ from most other dogs in their inability to convert uric acid to allantoin, and excrete over four times as much uric acid daily per unit of body weight as do other dogs. H. C. Trimble and C. E. Keeler[10] showed that this characteristic is determined by a recessive mutation not linked with the spotting characteristic of the Dalmatian breed.

For the most part, the great numbers of diseases described in Osler's book classify the data as follows: etiology; pathology (where known); symptoms; prognosis; diagnosis; treatment. For some diseases prophylaxis and epidemology are given, and only rarely pathological physiology, although from the latter the clinical syndrome emerges at higher structural levels. A classification of diseases based on physiological abnormalities traced to biocatalyst lack or deviation might be helpful not only in understanding and treating diseases, but also in understanding the mode of action of drugs which in many cases affect biocatalysts directly. Apparently no such classification of diseases exists, and the following resume is given of a few instances where existing knowl-

edge is sufficient to permit us to refer a disease or symptoms of an ailment to abnormal catalyst conditions.

To illustrate how extensive may be the varieties of chemical and enzymic attack on an organism following infection by a single agent, Dr. John E. Blair, of the Hospital for Joint Diseases, kindly prepared for me an outline regarding the toxins and enzymes or enzyme-like substances (catalysts) generally liberated by *Staphylococci,* the common cause of boils, carbuncles and similar troubles. The following is based on this outline.

Toxins and other products of Staphylococci

Toxins

(1) *Exotoxin.* This soluble, filtrable, thermolabile product can produce demonecrosis, hemolysis, and death, and may be a mixture of "lethal toxin," necrotoxin, and hemolysins, mentioned below.[11]
(2) *Necrotoxin.* Produces intense necrosis on intradermal injection, liberating leucotaxine which initiates blocking off of the infected area.[12]
(3) *Lethal Toxin.* Venous injection quickly kills mice and rabbits. Mice killed in 2 to 4 minutes by 0.05 ml.[13]
(4) *Enterotoxin.* Soluble, filterable, thermostable; responsible for food poisoning due to staphylococci.[14]
(5) *Leucocidin.* Destructive for leucocytes, often causing their complete disintegration. Produced in varying amounts by most pathogenic staphylococci, especially those responsible for furuncles and severe infections.[15]

Enzymes

(1) *Alpha-hemolysin.* 0.002 ml often hemolyses 1 per cent rabbit erythrocytes in 1 hour at 37° C. Little or no action on human red cells and apparently not harmful to humans, though produced by many Staphylococci harmful to man. Useful in estimating the potency of exotoxin, whose titer it closely parallels.[16]
(2) *Beta-hemolysin.* Hydrolyzes sheep and human cells, but not rabbit cells. Found in animal, and occasionally in human strains. No apparent relation to pathogenicity.[17]
(3) *Gamma-hemolysin.* Affects rabbit cells; no evident pathogenicity.[18]
(4) *Spreading Factor.* Increases permeability of connective tissue, until negatived by leucotaxine action.[19]
(5) *Coagulase.* Induces clotting of glood plasma and fibrinogen solutions. Found only in pathogenic staphylococci, and useful in their laboratory identification.[20]

(6) *Fibrinolysin.* Can cause lysis of fibrin clots. Found only in pathogenic strains; but action is much slower than fibrolysin found in hemolytic streptococci.[21]

Inflammation. Professor Valy Menkin of Temple University finds that inflammation is characterized by the formation of various common denominators, which are liberated from cells previously injured by an irritant. The following substances have thus far been identified in inflammatory exudates:

(1) *Leucotaxine.* Seems to be a peptide, possibly with attached prosthetic group. It increases capillary permeability and leucocyte migration.

(2) *Leucocytosis-promoting Factor.* Protein in character, or associated with proteins. It induces discharge of immature leucocytes from the bone marrow, where it also causes marked hyperplasia of granulocytes and megakaryocytes.

(3) *Necrosin.* A toxic material in the euglobulin fraction of the exudate. It enters the general circulation and can damage essential structures. Possibly it is a proteolytic enzyme.

(4) *Pyrexin.* Apparently a hydrolytic product formed by necrosin or associated enzymes. Separable from the euglobulin fraction. It produces fever, probably via the hypothalmic heat-regulating nerve centers.[22]

(5) *Leucopenic Factor.* May be only a separate factor in pyrexin, but seems to be a polypeptide when separated from pyrexin or otherwise recovered. Apparently it leads to rapid trapping of leucocytes in the alveolar walls of the lungs, in the sinusoids of the liver, and in the spleen.

(6) *Dextrose.* Formed on protein breakdown. In diabetics, this may enhance the diabetes, when there is a superimposed infection.

The emergence and the extent of these various factors in the syndrome of inflammation varies with the nature of the irritant and its amount, and with the animal involved. The degree of injury done to the cells varies, even in individuals of the same species. The view here maintained is that these specific substances owe their origin to interference with normal cellular catalysis. While some catalyst changes may be reversible or recoverable, others may be irreversible or irreplaceable, and may lead to the death of the cell and the chemical breakdown of its constituents. Pus contains many dead leucocytes in addition to necrotic material, as well as living and dead bacteria.

It must not be supposed, however, that the catalytic or enzymic aspects of diseases represent isolated entities that can be dissected out from the other related facts. For example, interference with the blood supply to cells, whether brought about by mechanical

constriction (strangulation) or by reduction of the lumen of arterioles (by arteriosclerosis) or capillaries (by drugs or by hormonal or nervous imbalance), can result in diminished oxygen supply and delayed removal of metabolic products. The normal oxidative breakdown of glucose may thereupon veer toward anaerobic fermentation whereby lactic acid is released. The consequences in the various cells and organs will no doubt differ considerably, as will also the secondary consequences throughout the body; but in general, lack of oxygen will upset the normal function of the cellular enzyme systems, even if it does not lead to enzyme inhibition or even to cellular death. During violent exertion, for example, in a 100-yard dash, some of the muscular energy comes from anaerobic breakdown of glucose. The "oxygen debt" of the tissues is gradually restored by accelerated breathing or panting.

As above mentioned, many diseases have a genetic basis, and involve inherited enzyme abnormalities, or are established by structural abnormalities consequent upon abnormal embryological development. In his recent book (1941) on "Medical Genetics" Prof. Laurence H. Snyder of Ohio State University points out that the familiar 3:1 ratio of Mendel no longer covers the major portion of the field of heredity, for geneticists have demonstrated varied types of heredity transmission—dominant, recessive and blending—as well as varied relationships and behaviors of genes, e.g., autosomal, sex-linked, sex-influenced, lethal, epistatic and combined genes as seen in multiple allels and multiple factors. Epistasis, in Mendelian inheritance, means "the expression of one character to the exclusion of another not of the same allelomorphic pair." Thus in rabbits and mice the factor for gray, if present, prevents the development of the factors for black and chocolate. This recalls the fact that some plants lie prone ("lazy" corn) or develop chlorophyll-free areas ("variegated"), probably because of the development by them of catalysts whose activities lead to these results. Many traits are transmitted by heredity, even though we have thus far been unable to unravel the precise mechanism. Thus strains of Dalmatians ("coach dogs") have been selected and bred which take definite positions—some close to the horse, some beneath the wagon, and some behind it.[28] Identical twins generally develop like heritable diseases and show like reactions on exposure to other diseases.

The following are mentioned by Snyder as among the many diseases transmitted by heredity:

Eye and Ear: albinism (the eyes are pink, since lack of pigments permits the blood color to come through); color blindness; deaf-mutism.

Skin: icthyosis; absence of sweat glands; beaded hair (which breaks off, leaving bristle-like pieces); Von Recklinghausen's disease (due to a dominant gene), a neurofibromatosis affecting skin and nerves; Mongolian spot.

Skeleton and Muscles: polydactyly (extra digits); syndactyly (digits joined or "webbed"); "crab-claw" and like imperfections; club-foot; myotoma congenita (Thomsen's disease); myopathy (progressive muscular dystrophy).

Blood: hemophilia; anemia; troubles due to blood groups, including Rh factor.

Nerves: a variety of nervous conditions, including insanities and Huntington's chorea. The violent involuntary movements in this disease generally appear only in adult life, and follow degenerative changes of ganglion cells of the frontal cortex and of the cordate nucleus and putamen.

C. B. Davenport states[24] that traits known to have a chemical basis have the cleanest-cut genetics, *e.g.,* blood groups, cretinism (thyroid deficiency), amurotic family idiocy (a recessive associated with the storage of phosphatides). "The relation between the somatic expression of a trait and its chemical basis may be remote. Thus hardness of hearing seems to depend on a defect in calcium metabolism such as causes abnormal bone formation in the oval window of the inner ear and simultaneously in other parts of the temporal bone. In the latter case there is reason for concluding that the result depends on a dominant factor in an autosome which modifies the reaction of the mesenchyme and a sex-linked gene which perhaps affects calcium metabolism. Indeed it seems probable that in time chemical errors in the body may throw light upon the chemical processes of development."

A rather rare kind of recessively heritable metabolic error is *alkaptonuria,* first described by von Boedecker,[24a] and recently observed in an American Negro family.[25] In these cases the urine contains homogentisic acid, shown by M. Wolkow and E. Baumann[26] to be 2,5-dihydroxyphenyl acetic acid, which is labile and oxidizes, turning the urine black. Apart from occasional blackening of cartilage (ochronosis) no other symptom is evident. Homo-

gentisic acid apparently appears because of the blocking, at this stage, of the normal oxidation of tyrosine and phenylalanine, which are normal constituents of many proteins. Normal persons completely oxidize the acid, and perfusion through normal liver converts it into acetoacetic acid. It was shown by H. D. Dakin[27] that alkaptonuria patients can fully catabolize *p*-methylphenylalanine and *p*-methoxyalanine, which cannot form quinoid derivatives; he believes that inability to catabolize homogentisic acid is associated with increased formation of the quinoid intermediate from which it is derived.

Detoxication, the route whereby organisms rid themselves of certain poisonous substances, also varies with the changing catalytic and chemical nature of the animal. Thus fowls eliminate phenylacetic acid and its homolog, benzoic acid, by combining them with ornithine, while all mammals combine benzoic acid with glycine (amino-acetic acid) and eliminate it as hippuric acid. However, Sherwin and Thierfelder,[28] found that though the lower mammals, including monkeys, combine phenylacetic acid with glycine and eliminate it as phenaceturic acid, man couples phenylacetic acid with glutamine and eliminates it as phenylacetylglutamine. But experiments on the chimpanzee by Prof. Francis W. Power of Fordham University showed that this "man-ape" eliminates phenylacetic acid in the same manner as does man. Here too, as in the biochemistry of muscle, there is a definite relationship between chemical mechanisms and the position of the animal in the evolutionary or taxonomic scale.

In some cases disease may follow photosensitization after introduction of stranger molecules. As far back as 1897 O. Raab observed that small amounts of acridine exerted a lethal action on paramecia in the presence of light, though the same doses had no effect in the dark. Later, similar effects were seen with eosine, chlorophyll, etc., H. F. Blum describes[29] a number of diseases which develop in grazing animals (sheep, horses, cattle) when they eat certain plants. Among these are fagopyrism due to buckwheat (*Polygonum fagopyrum*); hypericism, due to St. Johnswort (genus *Hypericum*); and geeldikkop ("yellow thick head") caused by a South African plant *Tribulus*, popularly called "devil's weed." In geeldikkop the active material seems to cause liver injury and occlusion of bile, accompanied by retention of phylloerythrin, a chlorophyll derivative. This substance, accumulating in the skin, makes the animal light-sensitive and leads to edema of the head

and other places exposed to light. Blum believes that the only way to account for all the evidence is to assume that when a quantum of light strikes a dye unit in a substrate-dye combination, the dye portion is activated and thereupon transfers its activation to the substrate, which then reacts with oxygen and injures cell structure. The reaction is evidently of catalytic nature, for it continues steadily without continuous addition of dye.

Cancer*

From a clinical point of view cancer is a progressive growth of somatic cells which are abnormal, which invade and injure healthy tissue, and form abnormal structures that often become infected and break down. Cancer cells, breaking away from the main mass, may wander elsewhere in the body, and there establish new cancerous growths (metatases). Furthermore, many cancer cells duplicate themselves in tissue culture. It is therefore obvious that in cancer we are confronted with a heritable change in the somatic cell affected. The medical terms are in general descriptive, indicating the tissue involved; e.g., glioma (nerve); carcinoma (epithelium); sarcoma (connective tissue); nephroma (kidney), etc.

A much clearer understanding of the basic cause of this protean group of diseases emerges if we descend to the catalytic level of biological happenings. For catalysts (genes, enzymes, symbionts) dominate the chemical and physico-chemical changes which are responsible for morphology and physiological function. Mere heritable change in a cell is not alone enough to make it cancerous, for, as has been pointed out, differentiation and evolution both involve heritable catalyst changes in cells. It is the peculiar and harmful *consequences* of the heritable catalyst change that leads the clinician to make his diagnosis of cancer.

It has long been known that the development of cancers in man and animals is associated with exposure to certain influences, and, more recently, to certain definite chemical substances. Thus the natives of Kashmir, who often suffer from burns from a charcoal brazier (*kangri*) which they carry about their waist, are prone to develop *kangri* cancer. The British surgeon Sir Percival Pott observed that chimney-sweeps frequently developed scrotal cancers; and later it was noted that cotton spinners exposed to oils and workers in tar and aniline factories show a high incidence of can-

*See review by Professor Leo Loeb (Washington Univ.) in Vol. V, "Colloid Chemistry," 1944.

cer. The compounds dimethylaminoazobenzene (butter yellow) and *o*-aminoazotoluene can produce liver and bladder cancers in rats if given orally or subcutaneously, and 3,4,5,6-dibenzcarbazole (made by hydrogenating and condensing two molecules of beta-naphthylamine) causes proliferation of the gall ducts in mice. Seamen exposed to tar and to insolation, as well as sufferers from x-ray and radium "burns" are apt to develop cancer, though carefully regulated x-rays of certain wave length and intensity are now used in attacking cancers.

When butter yellow is fed to rats, biotin favors tumor formation, whereas riboflavin and allied vitamins oppose the carcinogenic effects of this azo dyestuff.[30] These findings are understandable on the view that biotin favors or contributes to the formation of a new, reproducible catalyst area in what becomes a cancerous cell, whereas selective adsorption of the vitamin material blocks this development. A somewhat analogous case exists in the antagonism between the sulfa drugs and *p*-aminobenzoic acid (PABA), later referred to in this chapter. The relations between PABA, inositol and pantothenic acid, in affecting the graying of the hair of black rats, are curious and devious. According to G. J. Martin[31] the absolute amount of PABA is not the important factor in this syndrome, but its ratio to pantothenic acid, which dominates the vitamin-synthesizing powers of the gastrointestinal flora in the rats.[32]

In 1915 Yamagawa and Ichikawa[33] produced cancers in the ears of rabbits by continued application of certain tars. In the valuable book[34] by Prof. Louis F. Fieser of Harvard University there is reviewed the fascinating story of the team-work of chemists, physicists, physiologists and others, which led to the isolation, from carcinogenic tar, of a previously unknown but potently active hydrocarbon, 3,4-benzpyrene, although it was present only to the extent of about 0.003 per cent. Synthetic 3,4-benzpyrene, prepared after its structure was clarified, is identical in its action with that obtained from tar. A few details of this important development may be mentioned. High-boiling tar fractions proved to be non-carcinogenic; but I. Hieger[35] observed that active tars and oils gave a characteristic fluorescence spectrum, with bands at 4,000, 4,180, and 4,400 Å. Since 1,2-benzanthracene was found to give similiar bands, J. W. Cook prepared a large number of allied compounds, among which 1,2,5,6-dibenzanthracene proved to be actively carcinogenic—the first pure substance of known

structure thus characterized. Finally, J. W. Cook, C. L. Hewett and I. Hieger[36] extracted from about two tons of active tar a tiny percentage of 3,4-benzpyrene; it produces epitheliomas in mice after about 5.7 months application of dilute benzene solutions, whereas 1,2,5,6-dibenzanthracene takes about 8 months to produce a like effect. Later it was found that methylcholanthrene, closely related to certain sex hormones and first synthesized by degrading desoxycholic acid (a bile constituent), is still more potent, giving cancers in about 5 months. These carcinogenic agents may cause cancers to develop in various tissues, *e.g.*, epitheliomas when applied to the skin, sarcomas when injected subcutaneously. Prof. Fieser stated:

"While proof is entirely lacking, it appears possible that many forms of cancer may originate in the metabolic production of methylcholanthrene or related substances from the bile acids, or perhaps from sterols or sex hormones, of the body. . . . Entirely unknown is the mechanism whereby certain hydrocarbons start normal cells on a career of malignancy. If chemical reaction is involved, the nature of the change is entirely obscure. . . . Possibly the molecular dimensions and the surface activity of the substances are as important as their chemical characteristics."

The view here being maintained is that the effects of carcinogens are due to their ability to produce heritable changes in biocatalysts, either by inhibiting or modifying existing catalysts, or by entering into the creation of new ones.

In some cases viruses are known to produce cancer (*e.g.*, Rous chicken sarcoma), but it is not yet clear whether the self-reproducing (autocatalytic) virus acts as a catalyst of itself, whether it induces changes in other biocatalysts, or whether both of these events simultaneously occur. In any event, the virus, whether catalyst or catalyst inhibitor or modifier, is continually supplied by its own autocatalysis. It can catalyze the formation of specific antibodies in the host fowl, and it may compete for, utilize or destroy substances essential to the normal catalysts or metabolism of the cell.

Since there is not even a suspicion of evidence that the trivial quantities of chemical carcinogens reproduce themselves, it appears that their specificities of chemical structure, reflected in their active surfaces, enter into the formation of new self-duplicating catalyst areas. This is the mechanism whereby the cell starts off on a career of malignancy. Where cancer cells reproduce

themselves in tissue culture, no steady supply of the original carcinogen itself need be furnished; but in some instances a steady supply of activating substance may be important or essential. Thus Dr. Charles Huggins of the Dept. of Surgery, Univ. of Chicago, stated[36a] that "it is possible by reducing the amount or the activity of circulating androgens to control, more or less but often extensively, far-advanced prostatic cancer in large numbers of patients. In this special case, androgen control seriously disturbs the enzyme mosaic of the cancer cells at least with respect to the important energy producing protein-catalysts, the phosphatases. As a contribution to the problem of cancer treatment, it is well to emphasize that any interference with an important enzyme system of a cell, normal or malignant, will cause in that cell a decrease in size and function." Apart from castration, the administration of female sex hormone (estrone), or of the chemically quite different diethylstilbestrol, could cut down the circulating androsterone; and I am informed that this type of treatment is being successfully used in prostatic cancer.

It may be noted that the tendency toward cancer development in older people may be related to chemical alteration of sex hormones or their precursors, resulting in formation of carcinogenic substances.

In this connection it is interesting to refer to another case where a steady supply of an activating substance has led to cancer-like developments. Dr. Peyton Rous,[37] of the Rockefeller Institute for Medical Research, whose discovery of the Rous chicken sarcoma virus exposed a new type of cancer-producing agent, states*:

"Yet one discovery there was, made almost 40 years back,[38] which justified the inference—though this has only lately been drawn—that an actuating cause for neoplastic change need not necessarily originate within the cell. A certain few substances, notably the fat-soluble dyes Sudan III and Scharlach R, when dissolved in olive oil and injected just beneath the covering epithelium of the skin, will cause its cells to proliferate vigorously and invade the underlying tissue like those of a carcinoma. They may even penetrate into the blood and lymph vessels, though no secondary growths result. To all appearance an active epidermal cancer has come into being, but this state of affairs continues only so long as the stimulating influence of the dye permits. Within a few weeks, as the latter is carried away, the cells leave off aggression and differentiate like healthy epidermal elements, and soon

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all that remains is some scattered oil-containing cysts lined with ordinary epidermis. No real conversion into the neoplastic state has taken place, though a second injection of dye at the same spot causes malignancy to be simulated for a longer time. If the Scharlach R were not lost, if it went along with the multiplying cells, increased as they did, and because of this continued to urge them on, surely a growth running the course of a true neoplasm would result. Agents which do precisely these things have been discovered. They are the tumor-producing viruses."

There is evidence showing that the viruses may often persist in cells as harmless symbionts, ready to bring about disease when favorable conditions permit. An example of these so-called "latent" viruses occurs in the King Edward potato, where it causes no evident disease; but it can kill other varieties upon inoculation. And a virus infection superimposed upon a "pre-cancerous" irritation may cause rapid cancer development. Thus when the virus from the naturally occurring papilliomas of wild cottontail rabbits is injected into the blood stream of domestic rabbits already having papilliomas induced by tar, "it localizes in these growths, which are benign and usually indolent, with the result that some of them start growing with unprecedented speed, while others change equal swiftness to carcinomas, often very malignant, an alteration which tar papilliomas undergo only occasionally and after a long while if let alone. It is plain that the virus acts in concert with the unknown cause for tar tumors, with result in cancers which otherwise would not occur."[37] It must here be pointed out that the action of both the tar and the virus are explicable in terms of persistent and heritable changes in biocatalysts; the virus and/or its products may upset existing catalyst structures.

In certain strains of mice most of the old females develop breast cancers. If fathered by a male of a relatively "cancer-free" strain, the young of a "cancer strain" mother develop cancers. But the young of a "cancer-free" mother by a "cancer strain" father seldom develop cancers. It was found by workers at the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine, that in these cases this "maternal influence" is not genetic, but is transmitted to the sucklings by way of the milk. "So effective is the 'milk factor' that a single nursing during the first days after birth is enough to confer the liability to mammary tumors not only on the young but on their descendants. Whatever it is that the milk contains must get into the blood or lymph through the gut wall—which is especially permeable to large molecules during the first days of life—and be conveyed to the rudimentary mammary glands. Here it persists and increases in quantity as the glands develop.

At times it is demonstrable in the blood and in certain other tissues, but it causes no visible changes anywhere and has no untoward effect until the mouse begins to age. Then one or more cancerous growths appear. This happens earlier, and the tumors are more often multiple, in mice which have had many litters of young and in those injected with the sex hormones which stimulate mammary proliferation. From the cancers nothing can be secured which will cause tumors on direct inoculation, though they yield the 'milk factor' in quantity. The many physical and chemical studies made of this factor all go to show that it has the attributes of a virus, and it exhibits the narrow specificity of those which give rise to tumors, its presence ultimately resulting in growths of a single organ and of but a single sort, the kind of mammary cancer common in mice. Furthermore, antibodies capable of neutralizing it are formed by the body, as in the case of the tumor-producing viruses. Yet with all this it fails to cause growth when injected into mammary tissue."[87]

However, many specific molecules are known to lead to the development of cancers, *e.g.*, 1:2-benzanthracene and quite a number of its derivatives, besides other organic substances previously mentioned. While these carcinogens are not themselves duplicated in the cell or the organism, they give rise to autocatalysts which are duplicated, even in tissue cultures of the cells whose cancerous potencies they determine. It seems more reasonable to consider the few cases where viruses are known to produce cancers as special examples under the broader view of heritable cellular catalyst change, rather than to stretch the term "virus" far beyond its generally accepted meaning. Nor is it necessary to assume that "latent" viruses lurk in all plants and animal cells awaiting merely for a preliminary excitation before launching their cancerous onslaught. The experiments of Peyton Rous and W. E. Smith[89] are illuminating. Realizing that the period of gestation is too short for experimental production of mouse cancers *in utero*, they applied a mixture of the potent carcinogen methylcholanthrene and Scharlach R to embryo mouse epidermis inplanted in the thigh muscles of mice. The majority of the tumors that appeared were progressively enlarging, invasive carcinomas. By similar technique cancerous growths were produced in wide variety in lung, stomach, biliary tract, intestine, kidney, ureter, urinary bladder, ovary, testis, and nerve tissue. Consid-

ering these experiments, Rous states in the *American Scientist* (1946):

"To account for these multifarious neoplasms by the localization in the embryo tissues, before and after implantation, of tumor viruses like those already known, each capable of producing neoplasms of but a single sort, one would have to suppose that a great swarm of such agents circulate constantly in the blood of the mother mice or those to which the tissue had been transferred, and that they regularly lodge, each in the embryo cells suited to harbor it. Nature is profuse and often does things in what seems to be the hard way, yet the existence of such a state of affairs seems beyond belief. So, too, with the possibility that a single source virus reaches cells of every sort early in gestation, from then on sojourning with them and becoming peculiar to them, and that variants arise after awhile—if conditions are right—which manifest the narrow specificity and limited scope of the viruses with which we are acquainted. The milk factor can hardly be cited in support of this conception of a widely disseminated and pluripotential source virus, since its localization and its effects are both restricted to mammary tissue, while furthermore it is responsible for but a single kind of tumor out of the number to which the mouse mamma is liable. One is forced to conclude that the cells of the embryo have an inherent capacity to undergo neoplastic change."

Every cell, embryonic or not, has enormous numbers of specific molecules in extensive variety, and most kinds of cells may develop neoplasms. Besides carrying the specific molecules responsible for differentiation, the gametes or the egg may also carry particles (in some cases even viruses) responsible for neoplastic developments if suitable conditions permit the formation of cancer-determining catalysts.

There is reason to believe that the structures determining the specificities of some biocatalysts are, in part at least, held together by forces much weaker than those of primary chemical valency—by van der Waals forces, adsorption, or hydrogen bonds. That these forces are strengthened by desiccation and weakened by hydration is well known to all users of aqueous adhesives. Embryonic tissues are much more highly hydrated than adult tissue, and this might be a factor in their susceptibility to catalyst change. In the presence of suitable specific particles, new self-duplicating catalyst areas might form and lead to cancer. The phenomenon

of "crossing over" in the chromosomes bespeaks their soft, quasi-gelatinous nature, which permits them to break apart and re-cement themselves in new arrangements. Whatever truth there may be in the popular belief that a blow, on the tender and hydrous tissues of the breast, for example, may sometimes lead to a cancer, might become understandable on this basis of traumatic fracture of a harmless or useful catalyst area followed (fortunately rarely) by the formation of a harmful one. Experiments with localized and not too violent ultrasonic vibrations represent an experimental approach to such questions.

There is evidence that a virus, entering the body as an infective agent, may persist there and be transmitted to progeny, possibly forming a persistent addition to the self-duplicating units of the cytoplasm of body cells, or of the body fluids. Mice experimentally inoculated with a non-fatal dose of choriomeningitis virus develop an immunity in 5 to 7 days and rapidly become free of demonstrable virus. Pregnant mice when inoculated seem to pass the virus on to their offspring, which develop after birth almost as normal mice, but continue to carry the virus and pass it on to their progeny.[40] Dr. Charles Armstrong reported[41] that a strain of choriomeningitis virus had been passed "in an unbroken series from an infected mother through 11 generations of her offspring with no apparent tendency of the virus to weaken or disappear. It has also been observed by Haas[42] that these congenitally infected mice are far more efficient transmitters of the disease to normal cage mates than are mice inoculated after birth." Probably mice develop antibodies against the virus antigen. Furthermore, we have here evidence for a non-genic inheritance mechanism.

Plant Galls and "Plant Cancers"

Inoculation of a host plant with the bacterium *Phytomonas tumorfaciens* produces "crown gall." In the case of sunflower plants, true secondary tumors developed several internodes away from the primary tumor.[43] On confirming this work recently, P. R. White and A. C. Braun found that the secondary tumors were generally sterile, and their tissues could breed true *in vitro* in a medium which remained bacteria-free, even though capable of supporting a profuse growth of the gall-initiating *Phytomonas tumorfaciens.* These authors conclude that the affected tissues have undergone a drastic change, indicated (1) by the abnormal capacity to produce galls, and (2) by their abnormal growth habits

in vitro. "That this change was originally brought about by some stimulus from the crown gall organism seems clear. That its maintenance is not dependent on the continued presence of the bacterium is equally clear."[44]

These results are understandable on the basis of transmissible change in biocatalysts or catalyst systems, either through the modification of, or the creation of, self-duplicating catalysts, or through inhibitions of individual catalysts. Conceivably, these heritable changes may be carried by the genes or by the cytoplasm; but the original stimulus must form some new autocatalytic (self-duplicating) specific area or template.[45]

Fasciation

The commonest example of fasciation is the cockscomb (*Celosia cristata*), which came from Asia where the wild normal form (*C. argentea*) is widely distributed. Professor Orland E. White of the University of Virginia says[46] that "the term fasciation, like the term cancer, has been used to describe a series of phenomena, the most striking of which is regions of uncontrolled or unregulated and disordered tissue growth, resulting in an increase in weight and volume of plant tissue . . . When fasciation occurs in a specific structure, such as a branch, a stem, an inflorescence, an androecium or a root, it may remain localized in these structures, or it may occur as a general phenomenon fundamentally and simultaneously affecting the whole plant . . . Fasciation bears an analogy to cancer from the standpoint that apparently the same character is produced in a given organism by many different causes some of which are primarily and fundamentally environmental, while others are heredity mutations."

A species of tropical mistletoe (*Phorodendron*) seems to exist only in fasciated form. Fasciation is widely distributed geographically, climatically, ecologically and taxonomically, and is recorded from over 102 of Engler's vascular plant families. Though its effects in the growing point are most striking, it may occur in practically any part of the plant.

It is evident that quite a number of different kinds of chemical and physical initiators, some heritable, some environmental, may lead to the dreadful syndrome found in the clinical cancers. Science, through sanitation, engineering, chemistry, medicine, etc., is saving many lives; but as the average span of life grows longer,

the greater is the chance that one of the cancer-producing mechanisms may become effective. The view that cancers, basically, involve heritable changes in biocatalysts, has been fruitful in the case of prostatic cancer, and it may be helpful in finding preventive means for certain types. However, the problem is as wide as biological science and is progressing toward solution by work in many scientific fields, which should be encouraged. Help, and even cures for some cases, may come from unexpected sources. Meanwhile, surgery and other competent medical treatment are doing much. The far-sighted directors of research funds realize the importance of flank attacks on the cancer problem, and it is to be hoped that any legislative funds will not be limited to a direct frontal attack. Research is to be judged by results, rather than by the amounts of money given or spent; but it often takes time before the facts developed by research can be understood and applied.

Drugs

The Chinese Emperor Shên Nung (2838-2798 B.C.) is credited with having written the first Pên T'sao (Native Herbs), the three volumes describing 365 drugs. Modern editions of the Chinese pharmacopeia are much more extensive. In the reign of Djoser (Zoser), first Pharaoh of the Third Dynasty of the old Kingdom, who ruled from 2780 to 2760 B.C., the great architect and engineer Imhōtep did much to advance the healing art. In fact, the versatile Imhōtep, the Leonardo da Vinci of his time, became a legendary figure and was deified as the God of Medicine, a position later given by the Greeks to Aesculapius. Faith in the physician no doubt was an important factor, and it still is; but real knowledge regarding drugs grew then as it does now—by observing and recording results. Our more intensive, intelligent, and coordinated experimentation is termed research. However, even the most primitive peoples have contributed to the modern pharmacopeia: South America contributed quinine; North America, cascara sagrada; ancient China, stramonium, chaulmoogra oil, and ephedrine (*ma huang*). The African pygmies are said to pursue a huge elephant for days, peppering his hide with tiny arrows dipped in strophanthus, until this potent heart-poison brings him crashing to earth.

In more modern times, active chemical substances have been isolated from many natural drugs, such as, morphine, atropine,

and strychnine, and their actions appraised. Then experiments were made with chemically modified drugs. For example, in 1868 Drs. A. Crum Brown and T. R. Fraser found that by introducing a methyl group into strychnine, theobaine, or brucine, their tetanizing action was changed into a paralyzing one (Sir T. C. Allbutt). Then synthetic organic drugs appeared: antipyrine (1883), phenacetin (1887), acetylsalicylic acid or aspirin (1899), and salvarsan (1912). This last was known as "606," because that was its identification number among the many compounds being tested out by Professor Paul Ehrlich (1854-1915, Nobel prize, 1908), founder of modern chemotherapy, in his search for "magic bullets" which would kill pathogenic organisms but spare the patient. Strangely enough, Sir William Henry Perkin, whose chance discovery of the aniline dye Mauve opened up the synthetic dye industry, was actually trying to synthesize quinine. We still await its synthesis; but chemotherapy has meanwhile produced quinacrine (Atebrine), plasmoquin (Pamaquine), and plaudrine. The sulfa drugs are recent valuable synthetics, though sulfonamide was known about half a century before its pharmacological potency was revealed by G. Domagh (Nobel prize, 1939). In penicillin and streptomysin we have again relied on biological synthesis, though formidable technological problems had to be solved to produce the final drugs.

Many if not all drugs which are effective in small amounts operate on specific enzyme systems. Since much depends on purity of the drug, the dosage, the kind of animal tested, and many other conditions, it is not surprising that the literature is at times a bit confusing. Results of experiments made on isolated enzymes *in vitro* may not parallel what happens *in vivo*, where conditions are much more complicated. Much recent work has been summed up by Professor Frederick Bernheim of Duke University[47] and by A. J. Clark.[48]

The luminescence of various bacteria, fish, and insects (glowworm, fire-fly) is due to the reversible oxidation of luciferin (an organic compound) by an enzymic catalyst, luciferase.[49] Other conditions being normal (*e.g.*, state of culture, supply of oxygen and glucose, temperature, pressure), a growth of suitable bacteria tends to maintain a constant level of luminosity, which is affected by various drugs. Professors Frank H. Johnson and Henry Eyring of Princeton University found that sulfanilamide inhibits luminescence, acting as if the drug combined with a prosthetic group

of the enzyme. Drugs like urethane, alcohol, ether, and similar "lipoid solubles" seem to combine with bonds of the protein carrier, when reversible denaturization by heat or pressure renders these bonds available.[50]

Professor Clifford S. Leonard of the University of Vermont, in discussing the problem of the role of sulfhydryl enzymes in the mechanism of the action of certain chemotherapeutic agents[51] suggests that the action of mercurials and arsenicals is on protein enzymes containing SH groups. To be useful, the drug must adversely affect the catalysts of the invading organism, without too much harm to the host; so research now includes work on the biocatalysts of malarial plasmodia, trypanosomes, etc. According to von Brand *et al.*[52] 90 per cent of the respiration of the South American *Trypanosoma cruzii*, in cultures, is stopped by inhibitors of oxidases and dehydrogenases, and only 10 per cent by inhibitors of SH enzymes. The respiratory mechanism of this organism is therefore quite different from that of the African trypanosomes, and Chagras disease, which it causes, does not respond to drugs successfully used in the African type infections.

Referring to the work of Professor E. A. G. Barron of the University of Chicago and P. Singer,[52a] which aimed to determine "which of the enzymes are 'SH' enzymes and which enzymes are not dependent on the presence of free SH groups for their activity," Professor Leonard states:

"We can perhaps review Barron and Singer's work to advantage from a viewpoint which they did not discuss, namely the mechanism of action of arsenicals and mercurials on parasites, and on the host's tissues. For the concept that the essential attack of these agents is on an *essential enzyme system which is dependent on SH groups for its function*, or on multiple such systems, seems far more illuminating than the general attack on proteins or on glutathione. *RSH* becomes *ESH*, where E is the residual part of an enzyme. It would seem that far lower concentrations than those needed for such general attack would suffice to stop the intermediary metabolism of cells by means of this inhibition of enzymes. What processes would thus be stopped? Barron and Singer show that there are many SH enzymes.

"Thus in the first phase of metabolism of carbohydrate, SH enzymes include: muscle phosphorylase, phosphoglucomutase, hexokinases, and phosphoglycaraldehyde oxidase. In the stages: pyruvate to lactate, or to acetaldehyde and CO_2, carboxylase is an SH enzyme, lactic oxidase is not. In the oxidative phase of carbohydrate metabolism among the SH enzymes are: hexosemonophosphate oxidase, pyruvic oxidase

(and this is *very sensitive* to reagents), alphaglutarate oxidase, malate oxidase, succinoxidase, and the phosphorylases, myosin (adenosine triphosphatase) and myokinase.

"Not affected, and hence not SH enzymes, are: lactate oxidase, isocitrate oxidase, acid phosphatase, carbonic anhydrase, polyphenol oxidase and catalase, although they may have SH groups in their protein. SH enzymes of fat metabolism are: liver stearate oxidase, *E. coli* stearate oxidase, betahydroxybutyric dehydrogenase, acetate oxidase, pancreatic lipase; while among the enzymes concerned in protein metabolism, SH enzymes include: *d*-amino acid oxidase, transaminase, *l*-glutamic dehydrogenase and monamine oxidase, but not diamine oxidase, pepsin or trypsin.

"Barron and Singer concluded that the role of glutathione is to produce reactivation of SH enzymes where they have been rendered ineffective by oxidation or mercaptide formation. After all the years of controversy about the role of glutathione, this is a rather interesting postulate.

"Thus we see that there is such a multiplicity of enzymes which the arsenicals can attack, that we can scarcely put our finger on any one point of attack where we could say: this will damage the parasite before it will damage the host. One of the most sensitive enzymes was pyruvate oxidase. Should we consider that arsenical attack upon this enzyme is crucial for the parasites?

"Reiner and Smythe[58] showed in 1934 that *Trypanosoma equiperdum* splits glucose to two moles of pyruvic acid. Perhaps the amount of pyruvate oxidase which this parasite can form is limited, for late in trypanosomiasis in the rat there is a pyruvic acid acidosis in the host."

In a note Dr. Leonard points out that this pyruvic acid formation by *Tr. equiperdum* may account for its virulence in hosts, since L. Reiner and co-workers found that *Tr. Lewisi,* which many common gray rats may harbor in their blood as a low grade avirulent infection, splits glucose to succinic acid (4 C chain) and acetic acid (2 C chain), and both of these acids are readily handled by host tissues in processes which do not require the aid of the limited essential dietary factor, thiamine.

Immunological experience shows that antigens closely alike chemically may react with both their respective antibodies (cross reactions). Similarly, enzymes may in some cases attack chemically similar substrates (metabolites), and enzyme poisons or inhibitors may be anticipated or fended off by chemically similar substances which do not inhibit. The fact that para-aminobenzoic acid (PABA) commonly nullifies the antibacterial activity of sulfa

drugs,[54] led D. D. Woods[55] to the widely accepted view that the two substances compete for a bacterial enzyme system which uses PABA as an essential metabolite. But R. J. Henry[56] believes that PABA excludes the sulfa drug from some enzyme, probably the dehydrogenases or the protein co-enzymase systems.

The well-known ability of many pathogenic organisms to develop resistance to sulfa drugs, streptomycin, etc., indicates that they can form specific antibodies, or else develop catalysts which can destroy the drugs, or can become incapable of combining with the drugs, either by physicochemical change or by the formation of a protective layer.[57]

One of the most feared "poison gases" of World War I was lewisite ($Cl{-}CH{=}CHAsCl_2$), which poisons various catalysts. Peters, Stocken and Thompson[58] reported that dimercaptopropanol

$$\begin{array}{ccccc} OH & & SH & & SH \\ | & & | & & | \\ C & - & C & - & C \\ | & & | & & | \\ H_2 & & H & & H_2 \end{array}$$

known as BAL (British anti-lewisite) serves as an antidote. Among other things, it stops the toxic action of lewisite on the pyruvate oxidase system of the brain; and its selective affinity for arsenic and heavy metals enables it to reverse or detoxify the inhibition of SH enzymes by other metals. It has been used successfully against arsenic poisoning after intensive arsenical therapy for syphilis, and also in corrosive sublimate poisoning ($HgCl_2$), and in brass founders' fever (Zn). "BAL in oil for intramuscular injection should be stocked in every hospital pharmacy" (Leonard).

In World War II, a much feared but unused "gas" was di-isopropyl fluorophosphate (DFP), which irreversibly inhibits cholinesterase, the enzyme (or rather, group of like enzymes) that plays an essential role in muscular contraction and in the transmission of nerve impulses. There is evidence that rabbit and human plasma, blood cells, and tissues contain an enzyme capable of hydrolyzing the fluorophosphate, and much of this enzyme is contained in the liver. It may be seen that the degree of physiological symptoms or the death of the animal is a resultant of the two processes. The present data do not permit a quantitative evaluation of the role of each of these processes.[59]

Fluoroacetate of sodium, recently introduced as a rodenticide, kills many mammals, the results varying with the species; most commonly ventricular fibrillation or excessive stimulation of the central nervous system causes death.[60] The plant *Dichapetalum cymosum,* known to South African farmers as "Gifblaar" is reported by J. S. C. Marais to contain monofluoroacetic acid, and 1.0 to 1.5 grams per kilo of the dried plant is lethal for rabbits.[61] The "nitrogen mustards" (*bis* and

tris betachloroethyl amines) are nitrogen analogs of sulfur mustard (bis-betachloroethyl sulfide). Several enzymes, including hexokinase, are highly sensitive to nitrogen mustards. "However, the fact that there has been little correlation between the susceptibility of enzyme systems *in vitro* and *in vivo* has delayed the acceptance of the enzyme inactivation theory of nitrogen mustard intoxication."

Enough has been said to show the extensive and basic importance of the catalytic aspect in disease, in disease-producing agents, and in drugs helpful in curing disease. While an understanding of the underlying physicochemical basis of disease is not in itself a remedy, it should be helpful in efforts to avoid, ameliorate, and cure diseases, and it may light the way for research. However, we should always be ready to recognize and accept the unexpected and the unsuspected actualities.

When a drug irreversibly damages some essential catalyst of an invading organism, the organism dies. If the damage is reversible, the activities of the organism may be only temporarily inhibited. Where the catalysts affected are not vital ones, the organism may carry on what, for it, may be considered an abnormal life, and its usual forms may be modified. In this connection the facts regarding *Mycobacterium tuberculosis* and the closely allied *M. leprae* are of interest; their classical forms are the acid-fast rods which stain red in the Ziehl-Neelson technique (carbolfuchsin followed by acid-alcohol). Dr. Eleanor Alexander-Jackson of Cornell University[62] developed a differential triple stain for demonstrating and studying non-acid-fast forms of the tubercle bacillus in sputum, tissue and body fluids. The non-acid-fast forms, including hitherto undemonstrated zoogleal and globoid forms, are clearly brought out by this triple staining method, which can be summarized as follows:

The Ziehl-Neelson technique is first followed, including flooding of the slide with Loeffler's methylene blue counterstain; but the power of this blue stain is increased by mixing in, on the slide, 8 small drops of normal NaOH, and allowing this to remain not over one minute. The entire slide, apart from the acid-fast forms (if present) is stained deep blue. The washed slide is then flooded with a freshly made solution of sodium hydrosulfite (250 mg in 50 cc water), which removes the blue from all but the non-acid-fast tubercle forms. The slide is quickly washed and then counterstained with an acid green, and against the green background acid-fast red and non-acid-fast blue forms stand out clearly.[63]

Dr. Alexander Jackson has observed in cases of tuberculosis treated with streptomycin, and of leprosy treated with promin and chaulmoogra oil, that the rod form seems to be suppressed, while zoogleal and globoid forms predominate.

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Chapter 11

Catalysis as the Efficient Cause of Evolution

The basic notion of evolution goes back at least as far as the Greek philosopher Empedocles (450 B.C.). A little later Aristotle (384-322 B.C.) anticipated the theory of descent. The ancient Greeks, who acquired so much in art and practical skills from the earlier civilizations of Egypt and Crete, were no mere copyists, but transmitted to the modern world superlatively improved phonetic writing as well as great literary and artistic creations. But while Greek philosophy produced many ingenious intellectual explanations of physical happenings, these were seldom submitted to the touchstone of experiment. Archimedes (287?-212 B.C.), who devised experimental means for determining specific gravity, was the exception rather than the rule.

Henry F. Osborn ("From the Greeks to Darwin") gives the following resumé of Greek notions of causation: "The Greeks left the later world face to face with the problem of Causation in three forms: First, whether Intelligent Design is constantly operating in Nature; second, whether Nature is under the operation of natural causes originally implanted by Intelligent Design; and third, whether Nature is under the operation of natural causes due from the beginning to laws of chance, and containing no evidence of design, even in their origin."

In this epitome Osborn evidently confines his remarks to the first of the Aristotlian causes, which are:

(1) *Formal Cause:* the conception or idea of what is to be realized, whether this idea exists in the mind or in the nature of things.

(2) *Material Cause:* what is to be acted upon by (1).

(3) *Efficient Cause:* the force or agent that does the work.

(4) *Final Cause:* the object or end to be reached by the process.

We are here concerned largely with (3), the mechanism whereby the material substance of living things is so directed that there emerges the *échelle des êtres*, indicative of gradual evolution.[1]

Since what Osborn terms "natural causes" commonly operate under the laws of chance, there seems no experimental method of differentiating between the second and the third of the three causation forms he mentions, though many feel compelled by the wonders of natural phenomena as revealed by science, to believe in and admit the existence of something beyond human understanding. Apart from such metaphysical considerations, modern science tries to explain the causes of phenomena on the basis of the behavior of material units at various stages of structure or organization. This is real dialectic materialism, and is in itself most wonderful and interesting.

The materialistic political expansion of Rome left little room for scientific advance. According to Gibbon, the Roman Emperor Tiberius, on being shown a specimen of "unbreakable" glass, sent the inventor to immediate execution because the imperial coffers received taxes on glass.[2] The fall of Rome and the establishment of the Church as a factor dominating medieval thought reduced science largely to ecclesiastical quibbling under the fiat that the divine mind guided every detail in nature, a concept allied to the Mohammedan view: "It is the will of Allah."

The development of the Renaissance began to liberate science. Roger Bacon (c.1214-1294), the clear-thinking Franciscan who studied Arabic lore and dabbled with gunpowder, was imprisoned for 14 years. The protean Leonardo da Vinci (1452-1519), known to most for the great artist that he was, prided himself most on being an engineer, studied the flight of birds and sketched a flying machine. He also made keen anatomical and physiological observations and experiments, and pointed to the fossil shells found on mountains as evidence that those areas were once covered by the sea. His remarkable records, kept in "mirror writing," have only recently been published in part. Giordano Bruno (1549-1600) was burned at the stake as a heretic. But the world continued to move.

With the classification ("Systema Naturae") of plants and animals by the Swedish professor Carl von Linné (1707-1778), commonly known as Linnaeus, we begin to approach modern times. Georges Cuvier (1769-1832), who founded the sciences of paleontology and comparative anatomy, believed with Linnaeus that species were fixed by special divine creation, and so great was Cuvier's influence supported by that of Buffon (1707-1788), that when Jean Lamarck (1744-1829) advanced the view that there is a

progressive and imperceptible transformation of one species into another, the notion was rejected. Speaking of Cuvier, Prof. R. C. Punnett said: "Had it not been for him and his influence it is possible that Lamarck's views would have attracted more support, and that contemporary copies of 'Philosophie Zoologique' would not be among the rareties of zoological literature."

Unfortunately, Lamarck coupled his valid concept that animals become adapted to modes of life different from their ancestors, with what seemed a naïve theory of causation. Without attempting to explain the mechanism whereby the employment of the head in butting could lead to outgrowths of bone and epidermal horn in stags and bulls, he attributed the formation of these projections to "an interior sentiment in their fits of anger, which directs the fluid more strongly toward that part of their head."[3]

It was this vulnerable aspect of Lamarckism that led James Russell Lowell to include in the "Remarks of Increase D. O'Phace"[4] the following amusing lines:

Some flossifiers think thet a fakkilty's granted
The minnit it's proved to be thoroughly wanted,
Thet a change o' demand makes a change o' condition,
An' thet everythin's nothin' except by position;
Ez, fer instance, thet rubber-trees fust begun bearin'
Wen p'litikle conshunces came into wearin',—
That the fears of a monkey, whose holt chanced to fail,
Drawed the vertibry out to a prehensile tail ....

But the spirit of inquiry was in the air. The great Scotch-German philosopher, Immanuel Kant (1724-1804) in his "Anthropology," a book assembled in 1798 from lectures he had delivered during his long life, actually raised the question of man's origin from animals. Pointing out that when early man was surrounded by hostile wild animals, human infants, if they cried as loudly as they now do, would have been found and devoured by beasts, and that, therefore man must have been different then from what he now is, Kant wrote: "How nature brought about such a development, and by what causes it was aided, we know not. This remark carries a long way. It suggests the thought whether the present period of history, on occasion of some great physical revolution, may not be followed by a third, when an orang-outang or a chimpanzee would develop the organs for walking, touching, speaking, with a central organ for the use of

understanding, and gradually advance under the training of social institutions."

The great poet Johann Wolfgang von Goethe (1749-1832) was much interested in science, and observed, among other things, that the flower consists of metamorphosed leaves. In fact the term *morphology* was introduced by Goethe in 1817, and this branch of science has had much to do with the development of evolutionary theory.[5]

The foundations of comparative embryology were laid by Karl E. von Baer (1792-1876), who discovered the human ovum. His Biogenetic Law may be summarized (following Singer) as follows:

(1) In development, general characters appear before special ones.

(2) Less general characters developed from more general ones; then follow the special characters.

(3) As development proceeds, animals of one species diverge continuously from animals of another species.

(4) In the course of development, a higher animal passes through stages which resemble stages in development of lower animals.

His law of "corresponding stages," in the development of vertebrate embryos is exemplified by the story told of him that, when he had forgotten to label some jars containing specimens preserved in alcohol, he said: "I am quite unable to say to what class they belong. They may be lizards, or small birds, or very young mammalia, so complete is the similarity in mode of the formation of the head and trunk in these animals. The extremities are still absent, but even if they had existed in the earliest stage of development we should learn nothing, because all arise from the same fundamental form." Von Baer asked:[6] "Are not all animals in the beginning of their development essentially alike, and is there not a primary form common to all?"

Developments in the newer sciences of morphology, embryology and paleontology were steadily undermining the notion, acceptable to ecclesiastics and accepted by authoritative scientists, that species are fixed. Robert Chambers, a Scotch publisher, wrote (1843-1846) what became a very popular book entitled "Vestiges of the Natural History of Creation," which was published anonymously because he did not want to bring on his publishing firm the opprobrious charge of heterodoxy. In the course of his exposi-

tion, Chambers remarked that the existence of various domesticated races of animals indicates that at best certain species could become heritably modified. He also thought that the existence of rudimentary organs militated against the hypothesis of special creation, *i.e.*, small teeth in the fetus of the whalebone whale, tiny imperfect extra toes on the splintbones of horses, traces of hind limbs in certain snakes. As Punnett observes, the great popularity of "Vestiges" indicated that educated people were not averse to the notion of the mutability of species; but apart from scientific imperfections, the book gave no reasonable suggestion as to the process whereby a heritable succession of forms could be established.

Natural selection was the mechanism proposed by Charles Robert Darwin (1809-1882) and Alfred Russell Wallace (1823-1913) to account for this heritable succession, and they reached this conclusion independently—Darwin as a result of twenty years of study, Wallace during an attack of intermittent fever at Ternate in the Moluccas, in February, 1858, when thinking of Malthus's "Essay on Population" which he had read a few years before. This essay had also stimulated Darwin. When the idea of the survival of the fittest emerged, Wallace said that in a couple of hours he had thought out the whole theory. In three evenings he finished his paper, which he sent to Darwin for an opinion, and thus precipitated the publication of Darwin's work, first in a joint paper read to the Linnean Society on July 1st, 1858, and next year in "The Origin of the Species." P. C. Mitchell, Secretary of the Zoological Society of London, points out that the theory of natural selection, or survival of the fittest, had been suggested by William Charles Wells in 1813, and further elaborated by Patrick Matthew in 1831; but their pregnant suggestions remained practically unnoticed or forgotten.

It is interesting to note that Constantine Samuel Rafinesque wrote in 1832: "There is a tendency to deviations and mutations through plants and animals by gradual steps at remote irregular periods. This is a part of the great universal law of perpetual mutability in everthing." Born in Constantinople (Istanbul) in 1783, Rafinesque spent ten years in Sicily, and lost his extensive herbal and sea-shell collections together with all his papers when shipwrecked on his way to America in 1815. Within the next 20 years he accumulated an herbarium of 40,000 specimens. He visited Audubon, taught foreign languages at Transylvania University (Lexington, Kentucky), and in

spite of his protean activities as scientist, poet, philosopher, author, etc., he died in dire poverty in 1840 in Philadelphia.[7]

Another early American evolutionist was James Lawrence Cabell, Professor of Comparative Anatomy and Physiology in the University of Virginia, to whose book[8] Professor Charles M. Blackford has called attention.[9] It contained much in common with the views of Darwin which appeared a few months later.

The powerful support given Darwin's carefully documented views by Sir Charles Lyell, Sir William J. Hooker and Thomas Huxley led to their speedy acceptance by scientists. But ecclesiastical opposition, focused on a defense of dogma, was immediate, violent and persistent, and its repercussions appeared some years ago in the Stopes case,[10] involving a law passed by the State of Tennessee to prohibit the teaching of "evolution" in state-supported institutions. For centuries orthodox chronology was based on the "Annales" (1650-1654) of the English Archbishop John Ussher, who calculated from biblical data that the Creation began on Sunday, Oct. 23rd, 4004 B.C., and ended at sundown six days later, after the creation of animals and of man. But archeology has found that the ancient Egyptians had a well-developed civilization about 6000 B.C., and science has credible evidence that the earth is about two billion years old. The Proterozoic era is estimated at 500 million years, the Mesozoic at 125 million years, and the Cenozoic at 60 million years. Evidences of man appear in the early Quaternary, and may go back to the Pliocene epoch of the Cenozoic.

Today, however much scientifically informed people may differ as to the relative value of the various factors influencing evolution, they are unanimous in accepting evolution as a *fact.* It will be noted that natural selection and the survival of the fittest operate at what may be termed *macro-levels* of organization, involving not only competition between various *groups* of plants and animals, but also their adaptability to changing climates and other natural conditions. On the other hand, unless heredity could be depended upon to carry on the genetic and other changes which occur at *micro-levels* of organization or structure, *natural selection would have nothing upon which to operate.* Therefore, while fully recognizing the existence and operation of many aspects and factors governing the evolution of populations over the long past, we must confine ourselves here to the more basic micro-level, or ultramicro-level of *catalysis,* from which emerge all the heritable

structural and physiological changes that underlie the happenings at macro-levels. How complex these happenings may be, can be seen, for example, from the papers of Professor Sewall Wright,[11] from T. Dobzhansky's book "Genetics and the Origin of Species" (1941) and from G. G. Simpson's book "Tempo and Mode in Evolution" (1944).

We have no space here to enter these interesting fields of ecology and paleontology which deal with a very real phenomenon —natural selection. But as Sir Wm. Bateson remarked, *its function is to select,* preserving beneficial and destroying harmful changes, and remaining indifferent to neutral ones. Bateson also stated:[12] We cannot see how the differentiation into species came about. Variation of many kinds, often considerable, we daily witness, but no origin of species. But that particular and essential bit of the theory which is concerned with the origin and nature of *species* remains utterly mysterious. The claims of natural selection as the chief factor in the determination of species have consequently been discredited. Our doubts are not as to the reality or truth of evolution, but as to the origin of *species,* a technical, almost domestic problem."[13] Bateson became a geneticist "in the conviction that there at least must evolutionary wisdom be found." Actually, the basic problem may be put thus: By what material mechanism are heritable changes in bionts produced and transmitted?"

Our answer to this question is simply expressed: *The primal cause of evolution is a heritable change in existing and potential* biocatalysts,* leading to heritable changes in form, function and behavior, and therefore to heritable changes in reaction to and interaction with the surrounding conditions of life. Some of these conditions affect the living unit as an individual, for example, ability to secure and survive on existing food, and to accomplish successful mating. Other conditions affect principally groups or populations, *e.g.*, the existence of adequate food and group competition for it, geographical factors involving isolation, and ecological factors involving attrition between groups (red squirrels vs. grey squirrels, carnivores vs. herbivores). Another set of conditions upon which living things depend involves the presence in air, water or soil of many essential substances and the absence of

* This refers to the transmission of new, or increased amounts of, specific molecules which may in later development modify existing catalysts or help form new catalysts.

detrimental substances. Since most living things depend upon molecules which must reach them through the living or dead bodies of other bionts or through biological wastes or excreta, it is obvious that, from the chemical standpoint, few existing individuals or groups can live alone. But underlying all these and other complexities is the basic fact that evolution depends upon *heritable changes in biocatalysts,* the successful ones tending to survive, the unsuccessful ones to die out.

The record of the rocks, imperfect as it is, represents what actually *has* happened over millions of years with many widely scattered populations of plants and animals, only some (probably few) of which have left discovered fossil remains. It is impossible to obtain an exact picture of the ever-varying conditions in geologic times, though we have evidence of great movements in the earth's crust, of glacial periods, of the probable existence of an atmosphere containing more carbon dioxide than ours. Pointing out that the great defect of paleontology is that it cannot directly determine any of the cryptogenetic facors instrumental in the evolution of populations, because we cannot experiment with fossil animals, G. G. Simpson states:[14]

"On the other hand, experimental biology in general, and genetics in particular, have the grave defect that they cannot reproduce the vast and complex horizontal extent of the natural environment and, particularly, the immense span of time in which population changes really occur. They may reveal what happens to a hundred rats in the course of ten years under fixed and simple conditions, but not what happened to a billion rats in the course of ten million years under the fluctuating conditions of earth history. Obviously, the latter problem is much more important. The work of geneticists on phenogenetics and still more on population genetics is almost meaningless unless it does have a bearing on this broader scene. Some students, not particularly paleontologists, conclude that it does not, that the phenomena revealed by experimental studies are relatively insignificant in evolution as a whole, that major problems cannot now be studied at all in the laboratory, and that macro-evolution differs qualitatively as well as quantitatively from the micro-evolution of the experimentalist. Here the geneticist must turn to the paleontologist, for only the paleontologist can hope to learn whether the principles determined in the laboratory are indeed valid in the larger field, whether additional principles must be invoked, and if so, what they are."

The difficulties here expressed are rather of a semantic nature. The word "evolution" subsumes a great number of physical happenings at a variety of structural and organizational levels, extending from the atomic and molecular, to plant and animal groups having continental and even world-wide distribution. It is seldom possible to think of and reason with phenomena emerging at any one level, in terms of the structural or organizational units of some other level, especially if it be a remote one. Thus the farmer understands crops in terms of choice of seed, planting, cultivation, weeding, fertilizers, water and weather conditions, etc., without even knowing anything about the chemistry or genetics involved, though these underlie his success or failure. Similarly, chemistry and genetic factors underlie the various evolutionary phenomena emerging at higher levels. Since these factors operate through heritable changes in biocatalysts, we may justly consider such biocatalyst changes as the basic or *primal* cause of evolution.

What catalysts undergo heritable changes or additions? Not so long ago geneticists would have answered: Heritable change is due to gene mutations. Later, because of the "position effect," the word "mutation" was extended to include certain heritable chromosomal abnormalities affecting gene action. Dobzhansky states[15] "In a wide sense, any change in the genotype which is not due to recombination of Mendelian factors is called mutation. In the narrower sense, it is a presumed change in a single gene, a Mendelian variant which is not known to represent a chromosomal aberration."

The inheritance of non-chromosomal and non-genic catalysts is still under investigation, and the importance of cytoplasmic inclusions has been already mentioned. It is generally admitted that the particulate units known as plastids, found in plant cells, are independent constituents of the cell in regard to heredity. Viruses are also known to live in the cytoplasm of some cells, and to be transmitted from one generation of cells to another. The cytoplasm of the gametes must also contain specific particulate units which govern differentiation. Physical or chemical changes in any of these cytoplasmic inclusions may persist and may be carried by heredity; and so also may *the results* of addition of new or stranger molecules, as is the case in cells treated with cancer-producing substances (*e.g.*, 3:4-benzpyrene), or with yeasts which become "trained" to ferment new sugars.

What heritable catalyst changes are effective in evolution? The answer here must be, Only those which persist and give a biont (plant or animal) some heritable advantage over competitors in the struggle for life, under the conditions existing at the time and place at which the particular heritable catalyst change occurs. Many catalyst changes are lethal, or else produce sterility or low rate of reproduction; others are of no evolutionary importance. Many bionts possessing promising heritable catalyst changes may succumb to the perils of surrounding conditions, and only the survivors among these advantageous changes live and establish clones or families, which may then become factors in evolution at higher organizational levels where the struggle for existence is visibly waged.

The case of ascorbic acid (vitamin C) is in point. Many animals (dogs, cats, rats, fowls, rabbits) can synthesize this antiscorbutic factor, and do not develop scurvy if it is missing in their diet; and they can survive the long winter when natural greens are scarce or lacking. But monkeys and guinea pigs readily develop scurvy if deprived of ascorbic acid, and A. Szent Györgyi (Nobel prize, 1937) comments[16] that these two experimental animals "come from the ever-green tropical surroundings where there is green food (which contains ascorbic acid) all year round. One of the basic laws of Nature is laziness; Nature will not do unnecessary things, and if a species has no need to make ascorbic acid, it will not make it; it will forget, or never learn to make it. The rabbit cannot permit itself to do this, for it would die during the long winter in our moderate climate." Obviously if an animal's catalysts cannot synthesize ascorbic acid and this is essential to its life, the animal in nature is limited to areas where adequate vitamin C is found in foods naturally available. The habitat of such an animal could be greatly extended if it became independent of dietary ascorbic acid. This could happen in a variety of ways; by a gene mutation; by acquisition of a messmate or symbiont which can produce it (as is the case with many ruminants*); or by developing catalysts that can synthesize it.

The notion that all heritable change must be genic may sterilize thought, but will not affect nature. Dr. Francis B. Sumner[17] states: "It seems obvious that any one who sponsors a theory of evolution by large steps must provide an agency through which functionally related parts are brought to vary together in such a

* Cf. the case of the termite and of the lichen (see Index).

way as to insure the harmonious co-operation of the parts affected. That neither genetic linkage nor the manifold effects of single genes affords a mechanism for such correlation, to more than a very limited extent, will probably be readily granted. It is probable that such an agency, if it exists, must be sought in the field of developmental physiology rather than that of genetics." In physiology catalyst formation and/or modification is a most potent factor, even though not the sole factor.

We must also remember that what Sumner terms "large steps" have usually taken very long times under unknown conditions, and that our general experimental inability to induce corresponding changes by "accelerated" tests done within a few generations, cannot controvert the existing paleontological data. Henry Fairfield Osborn,[18] in summing up the results of over fifty years of study, states: "The evolution of the Titanotheres has little to say about physical or chemical adaptations, but it gives us the most thorough and profound insight we have ever gained into mechanical adaptations." Osborn then lists twelve "principles" of adaptations *at the visual or mechanical level of structure,* and concludes:

"Whatever may be true as to fortuitous mutation and as to chance or random variation in chemical and physical adaptations, the mechanical evolution of the Titanotheres, a unique record of ten million years in the development of the Titanothere germplasm, shows absolute continuity in every single organ examined. There is not the slightest trace of discontinuity or of random origin. There is a firm undeviating orthogenic* order in the entire animal mechanism. There is a phytogenetic continuity of germinal adaptation and reaction in response to secular changes of habit and of environment."

To a book entitled "Creation by Evolution,"[19] twenty-six outstanding scientists contributed papers covering many aspects of evolution. The Editor states that the book does not attempt to explain the origin of life, or to determine the causes that lie behind the changes in living things from age to age. It attempts to show that there are changes and to describe how they come about. Though the various authors are unanimous in their demonstrations that evolution is a fact, not a single one of them sug-

* Orthogenesis is defined as "the doctrine that the phylogenetic evolution of organisms takes place systematically in a few definite directions and not accidentally in many directions; determinate variation; a theory of evolution propounded by the German naturalists W. Haacke and T. Eimer" (Standard Dictionary).

gests any casual mechanism at the physico-chemical level. The index has one entry under "Evolution, causes of," which refers to a section of Prof. E. G. Conklin's paper "Embryology and Evolution," entitled "The Causes of Development and Evolution," where Conklin states:

"The causes of the development of an individual or of the evolution of a species are twofold, internal and external. The internal causes are represented by the organization of the germ cell, the external by surrounding conditions; the internal causes may be called heredity, the external causes environment. . . .

"The character of development depends primarily upon the nature (that is, the heredity organization) of the egg concerned, and secondarily upon the environmental stimuli. The former determines all the possibilities of development and its main course; the latter determines which of these possibilities are realized and modifies more or less the course of development.

"Entirely similar causes are at work in the evolution of races or species. With true insight Charles Darwin wrote many years ago: 'Although every variation is either directly or indirectly caused by some change in the surrounding conditions, we must never forget that the nature of the organization which is acted on essentially governs the result.' Whether these variations are first wrought in mature organisms and then transferred in some unknown way to the germ cells, as Lamarckians assert, or whether they first appear in the germ cells, as Weismann and his followers maintain, is a secondary, although important, consideration, into which we will not enter here. In conclusion it may be confidently asserted that the causes or factors of the evolution of the species and of the development of an individual are fundamentally the same."

The view here maintained is that catalysis is the basis of both individual development and evolution. However, all this deals with our origin, not with our destination, which is considered in the last chapter.

REFERENCES

[1] Incidentally, it may be mentioned that Aristotle was a surprisingly keen observer, even though he did record many erroneous notions of his time. In *Historia Animalium* (Book 4 Part I, Thompson's translation) Aristotle stated: "The octopus, by the way, uses his feelers either as feet or hands; with the two which stand over his mouth he draws in food, and the last of his feelers he employs in the act of copulation. . . ." Only recently has this observation been confirmed. J. T. Cunning-

ham states (Encycl. Brit., 11th ed. (1913) Vol. 19, p. 993): "One arm is always considerably modified in structure and employed in copulation, but it is only in three genera, one of which is Argonauta, that the arm spontaneously separates. The detached arm is found still alive and moving in the mantle cavity of the female, and when first discovered in these circumstances was naturally regarded by the older naturalists as a parasite. . . . Whether detached or not, the modified arm possesses a cavity into which the spermatophores are passed and the arm serves to convey them to the mantle cavity of the female."

[2] Pliny states (Historia Naturalis, Liber XXXVI, Par. 195) that under the reign of Tiberius the inventor's workshop was destroyed, but that even this story is not fully authenticated.

[3] Lamarck wrote "Phil. Zool." (1873) reprint, p. 254: "Dans leurs accès de colère qui sont fréquents surtout entre les mâles, leur sentiment intérieurs par ses efforts dirige plus fortement les fluides vers cette partie de leur tête, et il s'y fait une sécretion de matière cornée dans les unes (*Bovidae*) et de matière osseuse mélangée de matière cornée dans les autres (*Cervidae*), qui donne lieu a des protubérances solides: de là l'origine des cornes, et des bois, dont la plupart de ces animaux ont la tête armée."

[4] "Biglow Papers," First Series, No. 4, lines 31 *et seq.*, 1846.

[5] Goethe's "Naturwissenschaftliche Werke" occupy 12 volumes in the great Weimar edition of 126 volumes, including "Letters" (45 vols.) and "Diary" (13 vols.).

[6] "History of the Evolution of Animals," Vol. I, Leipzig, 1828.

[7] See his biography, by Richard E. Call.

[8] "The Testimony of Modern Science to the Unity of Mankind," copyrighted in 1858 and published in 1859 by Robt. Carter & Brothers, New York.

[9] "Popular Science Monthly," 187-8, Vol. 52, pp. 224-228.

[10] See "Let Freedom Ring," by Arthur G. Hays (H. Liveright, N. Y., 1928).

[11] "The genetical theory of natural selection, Journal of Heredity, 1930, 21, 349-356.

[12] In an address entitled "Evolutionary Faith and Modern Doubts," *Science* (1922) 55, 55-61.

[13] When two groups of plants or of animals become incapable of cross-fertilization, they would no doubt be recognized as separate species. Despite enormous differences in color, size, features, mentality, etc., the diverse races of *Homo sapiens* are cross-fertile.

[14] "Tempo and Mode in Evolution." (1944).

[15] "Genetics and the Origin of Species." (1941).

[16] Publication No. 14, *Am. Assoc. Adv. Sci.* (1940) p. 163.

[17] *Am. Naturalist* (1942) 76, 433-44.

[18] "Paleontology Versus Genetics," *Science* (1930) 62, 1-3.

[19] Edited by Frances Mason, Macmillan, N. Y., 1928.

Chapter 12

Philosophy, the Guide of Mental Life

The intimate relation between philosophy and science is evident from the fact that until comparatively recent times scientists were known as natural philosophers. Etymologically, philosophy means the love of wisdom. In its broadest sense, philosophy includes the physical and mathematical sciences, as well as mental and moral philosophy, now often called mental and moral science. Metaphysics (literally *after physics*) is a branch of philosophy which studies the first principles of being and of knowledge; and though it makes full use of scientific facts, it is often loosely spoken of as "speculative philosophy." Students of social phenomena want to be called "social scientists," for science to-day is a word to conjure with; but they are really social philosophers because they seek not only knowledge but also wisdom in social matters.

The social, moral, and political turmoil caused by the impact on mankind of numerous and basic discoveries of facts by experimental scientists brings into sharp focus the great difference between *scientific knowledge* and *philosophical wisdom.* Wisdom involves making proper and desirable use of what science reveals. The Duke of Wellington, addressing an officer whose report was replete with undigested details, blurted out: "Sir, your information is too great for your understanding." Stung into action by failure of statesmen and politicians to make wise use of the power scientific discoveries placed in their hands, many scientists are adding philosophy, politics, and statesmanship to their endeavors. Benjamin Franklin, an outstanding natural philosopher, was also a wise and capable statesman.

The main part of this book having been devoted to purely material matters, this last chapter considers some of the philosophical aspects and conclusions which a personal review of the impersonal facts of science seems to warrant. Since mathematics is largely used to develop new knowledge from experimental ob-

servations, it is necessary to stress the difference between pure and applied mathematics.

Pure mathematics is comprised of arithmetic, algebra, geometry, trigonometry, calculus, etc., while applied mathematics includes mechanics, astronomy, navigation, and physics (including molecular physics). Pure mathematics develops logical consequences from assumed facts—indeed it has been said that a pure mathematician is never so happy as when he does not know what he is talking about. But when we come to apply mathematics to any particular problem, we must be careful to start with proper facts and to apply suitable mathematical forms or formulas.

Scientific Facts and Mathematical Deductions

An essential preliminary to the application of mathematics to any problem is a statement of the facts and the assumptions involved. The facts are determined by such careful observations as we are able to make; the assumptions, either by our inability to determine the facts, or by the compulsions of mathematical expediency. The resulting deductions are frequently given the hallmark of "rigid or rigorous mathematical analysis," notwithstanding the assumptions or uncertainties on which they depend.

Take, for example, Stokes' formula or "law," which is widely used for calculating the fall of a sphere in a homogeneous fluid. While weighing the electron on extremely small oil droplets, Millikan found that Stokes' law failed, because to such tiny ultramicroscopic particles air was no longer a homogeneous fluid, and the drops fell more rapidly than the law indicated, through the voids between the molecules of the gases comprising air.

The often fruitful practice of mathematicians, physicists, and biologists of dealing with "fields" is a deliberately chosen form of escape mechanism, which enables scientists to deduce and explain the behavior of material units when complications of numbers, structures, and interactions become too great for the human mind to follow. The comparatively simple "three body problem," in general, is conceded to be insoluble; but mathematicians deal safely with four, five, and polydimensional space and with $\sqrt{-1}$, an imaginary quantity.

While throwing these sops to the mathematical Cerberus, we attempt, as far as our mentality permits, to envisage the physical mechanisms underlying phenomena. Energy may be correctly expressed by a formula, but we visualize it as due to material units in motion. When matter is "converted" into energy, as in

nuclear disintegration, neither matter nor energy is destroyed. The atomic nucleus apparently breaks up into smaller material units, most of which have high velocities and some of which have "negligible" masses. We continue to grope our way toward the demonstration of ever smaller material units.

Our senses operate by and through particulate units, and we are fortunate that certain threshold values of excitation must be reached before an effect is registered on our consciousness. It is a great protection to our nervous system that we cannot hear every sound, no matter how faint; but we can use microphones to amplify these sounds when we wish. The rushing circulation of the blood is not noticed by normal persons, but when the nerves are supersensitive it may be felt as a "thrilling" or "tingling."

Physical measurements are made by our senses, often assisted or fortified by some kind of apparatus. Most apparatus is so constructed as to outline a result on some visible graph or scale, whereon height, inflections, cusps, rate of change, etc., become visible and recordable. We thus avoid grave difficulties which are likely to appear if we rely solely upon the unaided senses of sight, taste, touch, smell or hearing. Thus among the 70 per cent to whom phenylthiocarbamide has any taste at all, some will find it sour, others bitter, others sweet—and these idiosyncrasies are heritable as Mendelian recessives. There is a real basis for the old Latin maxim *de gustibus non est disputandum.* Most human ears are deaf to sound vibrations in excess of about 20,000 per second; but mice can hear supersonics far beyond the human range. Bats ("bald mice" in French, and "flying mice" in German) guide themselves in dark caves by sensing the reflections from the walls of the supersonic squeaks they emit during flight.

The thimble-rigger,* the three-card monte man,* the pickpocket, and the magician, all know that the hand is quicker than the eye; and the many optical illusions known to psychologists show how careful we must be before assuming that seeing is be-

* The following definitions (Standard Dictionary) are for the benefit of guileless mathematicians, who are not always the calculating persons some might imagine:

Thimble-rig: A gambling game in which three thimble-shaped cups are placed upside down on a board with a pea or ball under one of them, and shifted about by sleight-of-hand, the aim being to induce bystanders to bet under which cup the pea or ball is; frequently played at race-tracks, fairs, etc., in a swindling manner.

Three-card monte: A sleight-of-hand game or trick played with three cards, one of which is usually a court-card, in such a manner as to deceive the eye of the onlooker, who is induced to bet that he can pick out the court-card. Called in England *three-card trick.*

lieving. Tests indicate that insects have a visual wave-length range different from ours; and human beings too have a relativity in sensitiveness to light, for example, color blindness or Daltonism, so called after the great chemist, who discovered it in himself. Buildings are erected on the sufficiently close assumption that plumb-lines are parallel, although we know that plumb-lines in Istanbul are about at right angles to those in New York.

There has been much controversy and probably also much fraud in connection with "dowsing," the art of locating underground streams of water with a "divining rod"; but so eminent a scientist as Sir J. J. Thomson reports in his autobiography, "Recollections and Reflections" (1938), that there is no doubt about the reality of the phenomenon, even though at present we have no explanation for it. Possibly it might be associated with the ability of some persons to respond to radioactive, electric, or other influences developed by flowing water (streaming potential). Electroencephalograms and electrocardiographs recording electrical impulses developed in our bodies, are matters of routine determination in many hospitals. Homing pigeons have been extensively used for years, but we have no idea as to how they manage to reach their home lofts from great distances.

The results which the physicist deduces by mathematical logic can never rise above the level of the accuracy of his data or assumptions—and there are limits to the accuracy of practical measurements. The plea of Portia for Shylock's death "if the scale do turn but in the estimation of a hair," is good drama, but bad law; for the law in an analogous case would allow a reasonable tolerance in weight. Engineers, however, generally insist that "reasonable tolerance" be expressed in specifications, which may, for example, call for rods having a length of five inches "plus or minus one thousandth." With ball-bearings, turbines, etc., closer tolerances are called for; and photo-cell devices and diamond-tipped calipers are used to avoid errors that might follow wear on less durable material.

Heisenberg's Principle of Indeterminism[1]

When we attempt to measure such basic things as the velocity of an electron *and* its location, we find that our very means of measurement influence the result. This has led physicists to accept Heisenberg's principle of indeterminism or uncertainty. In common language this principle states that there is a limit to

observational experiments, which is reached when the observational or determining factors begin to interfere with the normal happenings in the experiment under observation.

The uncertainty principle having demonstrated the impossibility of making critically accurate measurements at subatomic levels, modern physicists, as mentioned above, transferred their attention to "fields," which permitted them, in the words of Professor Charles Galton Darwin,[2] "to carry through a formal mathematical analogy without ever asking what it all meant in terms of observable things . . . There grew up a great cult of doubting the reality of unobserved things, and then a curious thing was found; the charm did not work again . . . The work of the new quantum theory has in fact run most surprisingly in the opposite direction. The technique is largely concerned with wave functions, which are much more abstract than anything in classical mechanics. There is certainly nothing observable, or even picturable, about waves propagating themselves in many-dimensional space with absolutely unknowable phase, and with intensity controlled by the curious extraneous rule of normalization. Largely by use of these wave functions the whole of atomic physics has been reduced to order, and so has molecular physics, except that it yields problems in which so many electrons are interacting that a full discussion is not feasible. So the doctrine of theorizing only about observables was really not a useful doctrine; it merely provided a geminating idea. In fact, we may well ask what an observable is, and if we go at all beyond direct sensations, which as physicists we certainly intend to do, the answer becomes perfectly indefinite. This opinion I heard admirably expressed by the late Professor Ehrenfest. It was in a physics meeting in Copenhagen and some one was proposing a way out of certain difficulties which involved, as he maintained, a reversion to the cult of the observable. Professor Ehrenfest said: 'To believe that one can make physical theories without metaphysics and without unobservable quantities, that is one of the diseases of childhood (*das ist eine Kinderkrankheit*).' "

Probability

Chemical and physical kinetics recognize the impossibility of predicting the behavior of *single* atoms or molecules, and base their calculations on the laws of chance and probability, just as do life insurance actuaries, who can never foretell just when any

one policy holder will die. With the Gaussian laws of chance, as with the Boltzmann entropy probability theorem, the more units we deal with, the more accurate our calculations; but they cannot be applied to a single unit. Since the behavior of individuals depends on myriads of non-calculable physical and physico-chemical contingencies, it is obvious that mathematical calculations regarding the behavior of any individual (if indeed such calculations could be made at all) would be tainted with elements of uncertainty.

The saying that marriage is a lottery has a physico-chemical basis. The potencies of inheritance, sex, hormones, vitamins, drugs, alcohol, dyspepsia, nervousness, suggestion, autosuggestion, etc., being what they are, social scientists, military leaders, and business men base their calculations on what may be expected of the average person. Business proceeds on the basis that most men are honest, but allows for shop-lifting, locks its doors at night, employs watchmen, and insures against theft. The Devil can always quote Scripture to his purpose; but over-stern judges should read Robert Burns' "Address to the Unco' Guid, or the Rigidly Righteous."

The Time Factor

Bertrand Russell wrote:[3] "If you put a kettle on a nice hot fire, will the water freeze? 'Certainly not,' says common sense, indignantly. 'Probably not', says physics, hesitatingly. According to physics, if every member of the human race put a kettle on the fire every day for the next million million years (during which, according to Jeans, the world is to remain habitable), it is not unlikely that sooner or later the water in one these kettles would freeze instead of boil."

But Russell failed to state that among the ever-changing concatenations of molecular agitation, the extremely rare situation that would permit freezing would last for *so short a time* that it is unreasonable to believe that any freezing could take place—certainly no demonstrable freezing. Similarly, a probability calculation would show that if we only wait long enough, a brick might float in air, because at a certain instant all or nearly all of the impinging air molecules would strike the brick on the under side, thus giving it an upward lift (at sea level) approximating fifteen pounds to the square inch. But this calculation should also indicate that this condition would be so fleeting that no one

would notice it. It would be succeeded by other equally fleeting instants where molecular bombardment would actually add to the fall of the brick.[4]

It will be observed that the principle of indeterminism as well as the deductions from probability calculations are generally applicable to the "same time" or a "given instant." However, an instant—mathematically, a point of time—has no more practical existence for us than a point in space. What we term the "present" is in reality the immediate past and the immediate future. The "present" has become the "past" before our sensory and intellectual apparatus can inform us what was then occurring, even when aided by clever mechanical devices.

Free Will

Theology long ago advanced the metaphysical notion of predestination or *predeterminism,* according to which an omniscient and omnipotent God has decided, for all eternity, exactly what will happen; and if everything was thus "foreordained," there is really no such thing as free will. The philosophical and scientific analog of predestination is the doctrine of *determinism,* according to which physical events, whether on the outside or in the brain, strictly necessitate the character of all human volition. If we accept this materialistic fatalism, probability and the physical principle of indeterminism (Heisenberg) make man a mechanical automaton, a helpless plaything of chance. On the other hand, the doctrine of predeterminism makes man a helpless tool in the hands of God or Fate (Kismet).

Rebellion against this impasse appears in the quatrains of Omar Khayyam:

We are no other than a moving row
Of Magic Shadow-shapes that come and go
Round with the Sun-illumined Lantern held
In Midnight by the Master of the Show.

But helpless Pieces of the Game He plays
Upon this chequer-board of Nights and Days;
Hither and thither moves, and checks, and slays,
And one by one back in the closet lays.

If man, like the billiard shark condemned in Gilbert's "Mikado," must play extravagant matches, with fitless finger-stalls,

on a cloth untrue, with a twisted cue and elliptical billiard balls, what becomes of free will?

Freedom of will is mental, not physical. What we call freedom of the will is essentially the self-imagined ability if a person to select, at a certain time and under certain conditions, a certain alternative of thought or action. The selection involves no infallible assumption or guarantee as to what the immediate or remote consequences will be in every case, and absolute uniformity of results is seldom achieved or expected. The ethical and moral criteria of the situation are met, and free will is exercised, when a choice is made or, to throw a sop to the pure mechanists who claim a material *force majeure,* when the chooser *thinks* he makes a choice. Descartes' dictum, *Cogito ergo sum,* indicates how basic are the unseen springs of our consciousness and reason, from which emerge *conscience* and the power to make choices.

The law has long recognized this situation. Criminal choice or intent (moral turpitude) is generally alleged in felonies. A common allegation in indictments is that the act was committed "wilfully, wickedly and maliciously, with malice prepense and aforethought." In some of the old English court records it was applied even against animal offenders. In order to obviate the difficulty of proving the putative state of mind of the law-breaker, laws often rehearse or assume that certain acts are in themselves presumptive evidence of criminal intent. It must be stressed, however, that the laws existing at any particular time represent merely what is put into effect by the dominant powers of the state; that these laws will differ widely from time to time and from place to place; and that, owing to legal and legislative time-lag, they often do not represent the current popular mores.

Thousands of years of human experience have established certain actions and inhibitions as desirable in human society. Thus the injunctions laid down in the Ten Commandments are generally accepted by civilized mankind. They seem, in part, to represent a condensation and extension of the forty-two items of the chapter, in the Egyptian "Book of the Dead," known as "The Negative Confession." Seven of these negative statements to the underworld Judges (*e.g.,* "I have not robbed with violence; I have not made light the bushel") represent forms of stealing which are condensed into the Eighth Commandment, "Thou shalt not steal." The statement, "I have not defiled the wife of a man," corresponds to the Seventh Commandment, "Thou shalt not commit adul-

tery." It is to be noted that many of the Ten Commandments are also in the negative form. In any event, moral and ethical codes reflect states of human and social development, which change from time to time and vary from group to group.[5]

In final analysis, a human being can actually carry out only two types of activity: he can think, and he can change the position of some material things. The phenomena which then follow are usually the resultants of the natural mental or physical forces which are thus set in motion; for the effects may be on the minds of other human beings, as well as upon mechanisms actuated by push-buttons or levers.

The immediate or the remote consequences of what an individual chooses (or thinks he chooses) to think or to do may not always be rigidly certain; but they are, as a rule, sufficiently certain to serve as guides for human behavior in the future, as they have been during the long past. Sciences of all kinds teach us to see more and more clearly what we may reasonably expect to be the consequences of our thoughts and choices of action. Both material and ethical progress lie along such lines. It may be recalled that when Jesus of Nazareth was being crucified, He said: "Father, forgive them, for they know not what they do."

Now it may very well be that certain configurations of physical units possessing the power of thought and of action would, if precisely duplicated and placed in precisely the same conditions, always respond with precisely the same thoughts and choices. But the law of probability indicates how vanishingly small are the chances for precise duplication of any complicated set of structural and milieu conditions. The particulate nature of all matter, including our scientific apparatus, our sense organs, nerves and brain, as well as the electrical and other forms of energy whereby sense impressions are received, transmitted, and integrated into concepts, leads to the conclusion that neither human senses nor mentality can secure accurate premises whereon to base any logical conclusions as to causality. "The principle of logic remains true that from given premises there always follows a unique conclusion, but it does not apply where there are no premises or not sufficiently accurate premises."[6]

Genetics teaches us, on the other hand, how powerful are the effects on thought and action of inherited physical structure. Identical twins, arising from the same fertilized ovum, are surprisingly alike in structure, action, and thought. Their finger-

print patterns and electroencephalograms are often difficult to distinguish.[7]

The similarities of physical structure and behavior just referred to are evident to other individuals, that is, to outside observers; but the *thoughts* controlling the actions of the individual himself involve what we term his *conscience,* and are directly known only to himself. Acts of individuals which are in accord with mores are regarded by others as ethical and moral. If consonant with existing law, they are legal. But thoughts, the secret springs of action, are dominant factors in the scope of choice we call free will. No matter how a person may be limited by his physical inheritance, and his social environment, he is ethical and moral (even if not legal), when he honestly follows his own "inner light." Education has important ethical and moral responsibilities. Wisdom should guide those who learn to think independently; but many are guided solely by what they are taught or by the example set by teachers, parents, or associates.

The essential difference between the material and the mental, ethical, spiritual or moral aspects of an action is illustrated by the story of the Scot who attempted to retrieve a half-crown he had dropped into the plate at church, thinking it was a penny, but was frustrated by the Verger, who quickly covered the plate with his hand and remarked: "Nay, nay, mon—in for ance, in for aye!" "Weel", said the Scot, "I'll get credit for it in Heaven." "Nay, mon," returned the Verger, "in Heaven ye'll get credit only for the penny ye intended to put in." (John Brown: "Rab and His Friends").

Ethics and morality are not inherent in molecules, atoms, or subatomic particles. They are, rather, emergent relations which come into being only with the development of complicated material structures capable of thinking and choosing. These powers we believe to be most highly developed in man, but they are foreshadowed in the lower animals. It is interesting to note how the poet's eye in glancing "from heaven to earth, from earth to heaven," has envisaged these relationships between the physical and the metaphysical. Thus in his poem "Caliban upon Setebos," Robert Browning pictures the self-centered reaction to natural phenomena of that low form of human mentality, "a freckled whelp, hag-born—not honour'd with a human shape":

'Thinketh, such shows no right or wrong in Him,
Nor kind, nor cruel: He is strong and Lord.
'Am strong myself compared to yonder crabs

That march now from the mountain to the sea;
'Let twenty pass, and stone the twenty-first,
Loving not, hating not, just choosing so.
'Say the first straggler that boasts purple spots
Shall join the file, one pincer twisted off;
'Say, this bruised fellow shall receive a worm,
And two worms he whose nippers end in red;
As it likes me each time, I do: so He.

The introspective mind of Rabbi Ben Ezra responds on a much higher spiritual level:

He fixed thee mid this dance
Of plastic circumstance,
This Present, thou, forsooth, wouldst fain arrest;
Machinery just meant
To give thy soul its bent,
Try thee and turn thee forth, sufficiently impressed.

In "Saul," Browning sees evidence of perfection everywhere in nature:

Do I ask any faculty highest, to image success?
I but open my eyes,—and perfection, no more and no less,
In the kind I imagined, full-fronts me, and God is seen God,
In the star, in the stone, in the flesh, in the soul, and the clod.

Resentment at the inability of humanity, despite choice, to control the inexorable powers of nature, blazes forth again and again in Omar's lines:

And this inverted bowl we call the sky,
Whereunder, crawling, cooped, we live and die—
Lift not thy hands to it for help for it
As impotently moves as thou and I.

Ah, love! Could thou and I with Fate conspire
To grasp this sorry scheme of things entire,
Would we not shatter it to bits and then
Remold it nearer to the heart's desire?

But in William Henley's "Invictus" we find a defiant trumpet-call of humanity to material limitations:

It matters not how straight the gate,
How charged with punishments the scroll;
I am the master of my fate,
I am the captain of my soul.

Not only are we limited in our efforts to make physical measurements and to understand the vast complexities of nature, but the very ultimates of both mind and matter continue to elude us. As Herbert Spencer said, they are unthinkable.

Science and Religion

Science and religion originated together as a duplex reaction of the developing human mind to the physical universe, and to the needs and yearnings of the individual and of family and social life. Contact with physical nature led to primitive sciences (skills, manufacture, agriculture, and the household arts), while at the same time fear, love, wonder and introspection gave rise to primitive philosophies and religions. Primitive man looked with awe, love, or fear upon the gigantic forces of nature, and the early "medicine man" was both scientist and priest, striving to understand nature and to propitiate the many potent man-like gods to whom a naïve animism attributed control of rain and crops, lightnings and storms, and the mysteries of life, illness, and death.

Science and religion have undergone and are undergoing parallel evolutions, and we must not scorn "the base degrees by which we did ascend." There is a wealth of wisdom concealed among the many errors of folk-lore.

A few examples may be mentioned. In No. 11 of a series of books on "Tales from the Outposts," there is a chapter on "The Savage as Scientist," from which the following is quoted:

"Kinga, who was one of the most famous chiefs and rain-doctors in East Africa, refused to be moved from his kraal at Mandi on the Daua Plateau down to Sekenke in the Wambare Plains, as medicine-man Mgendu urged: and Mgendu came to ask advice of the writer, who was then administrative officer in charge of the Iramba tribe. (Kinga was suffering from general paralysis.)

"Said Mgendu: 'The *vidudu* of paralysis must fight with the *pilintu* of malaria so that the *pilintu* may be devoured; then must Kinga eat of the *nzizi chungu* (bitter roots), and he will be strengthened' . . . *Vidudu* are mysterious insect-like things; a *pilintu* is a strange unknown wormlike thing . . . that half-naked savage doctor was prescribing the most up-to-date medical treatment for paralysis based on the most recent discoveries of medical science . . . Sekenke is one of the worst malarial districts in all Africa.

"Many tribes, not only the Masai and Nandi of Kenya, knew the cause of malaria. The Somalis knew, for a British traveller in their country was told by Somali tribesmen thirteen years before Ross's

discovery that the *kan'ad* or mosquito was a bad insect, biting a man and making his blood boil with fever. Chief Kitandu of the Iramba tribe knew four centuries ago, for his minstrels sang to the twang of the *lusembi*, a primitive calabash guitar, 'Ni aza kusengila pana nu imbu; nu imbu mbii masaka kasenkila!' (Do not build huts where mosquitoes live; for mosquitoes are evil, and make your blood hot!) And that song, with others full of savage wisdom, is to be heard to this day in the kraals of Tanganyika."

In 1927 Dr. J. Wagner-Jauregg received the Nobel prize in medicine for his discovery that malarial infection may be used to produce high fever, with which certain nervous consequences of syphilis may in many cases be successfully combatted.

Only a dozen years ago[8] the mistaken notion was advanced that the biochemical aspects of scientific medicine began in 1894 "when the use of thyroid gland in the treatment of myxodema was discovered." Professor Edward H. Hume, then at the College of Medicine at Changsha (Yale-in-China) stated:[9] "Organotherapy is described as early as the 6th century A.D., when sheep's thyroids were used for cretinism. The practice is familiar to housewives throughout the land."

The use of "ashes of sponges" as a remedy for goiter was common from the latter part of the 13th century, and was probably based on earlier folk lore. The disease was, of course, known in ancient times and is mentioned by Pliny. In 1812 Courtois discovered iodine, and seven years later Dr. Coindret, a well known physician of Geneva, walked into Le Royer's pharmacy there and asked the chemist J. B. A. Dumas (then only 19 years of age) to determine for him whether sponge, especially burned sponge, contains iodine. When Dumas reported that he found iodine, "Dr. Coindret no longer hesitated to consider iodine as a specific against goiter."[10] In 1833 Bouissingault suggested the addition of iodine to cooking salt, since he observed in South America that goiter was prevalent where iodine was lacking. Today iodized salt is widely used; but an excess of iodine must be avoided, as this, too, can cause trouble.

Through all the non-essential beliefs and rituals of early religions, there runs the basic attempt of the introspective mind and developing soul to reconcile the hard facts of experience with human life and love. Care for the dead is one of the lines of cleavage between men and brutes, and with it came thoughts of a hereafter. The embalmers of Egypt were of the priestly clan, which included the scientists also. Moses, educated in Egypt, outdid his teachers, who had followed him as far as bringing up frogs upon the land of Egypt; for it is stated in Exodus VIII, 18: "And the magicians

did so with their enchantments to bring forth lice, but they could not."

It is unfortunate that in the past most organized religions have magnified their differences, thus tending to obscure their basic unity in God. Much blood has been shed over unimportant differences of opinion or ritual, to the exclusion of the common belief that God is the Father of us all. But religions are slowly changing. Thus Jonathan Edwards saw hell paved with the victims of the Calvinistic doctrine of infant damnation; but the somewhat more humane Michael Wigglesworth (1631-1705) in his poem on the Last Judgment ("The Day of Doom"[11]), had God grant as a boon to unbaptized infants "the easiest room in Hell." Not until 1902 was this doctrine of infant damnation removed by vote from the creed of one of our prominent churches, whereupon a modernist member of the convocation moved that the vote be made retroactive!

Though much has been written and said about the supposed conflict between religion and science, such a conflict never really existed. The eternal verities of both religion and science tower unconcerned above the storms of creed and dogma, glowing in the light of ever-advancing scientific knowledge and true religious understanding. For many years scientific development was obstructed by the fear that demonstrable facts would render untenable the dicta of theologians, as witness the treatment accorded to Galileo, for example. Astrology, the ancient art of divining the future by observations of the heavenly bodies, goes back to the earliest Babylonian history (about 3,000 B.C.); but this pseudoscience of the early priests and magi did not begin to slough away from real astronomy until about the time of Isadore of Seville (died, 636 A.D.), and it was utterly discredited by the scientific revelations of the great natural philosophers, Copernicus and Newton.[1]

Science, too, has had to struggle for its advances. Thus Prout's hypothesis as to the basic origin of the elements from hydrogen, was supposed to have been completely disproved by the meticulous atomic weight determinations of Stas; but discoveries in this century by Curie, Rutherford, and others show that Prout was quite near the truth.

Mind and Matter

Although philosophy, which coordinates and appraises all knowledge, indicates that both material and psychic ultimates

are beyond the range of human conception, the daily life of every person demonstrates belief in and guidance by the actual realities of matter and of mind. Without attempting to explain them, the law deals with both matter and mentality. Ralph Waldo Emerson in his first publication (1836) entitled "Nature," said: "Philosophically considered, the universe is composed of Nature and the Soul. Strictly speaking, therefore, all that is separate from us, all which Philosophy distinguishes as the NOT ME, that is, both nature and art, all other men and my own body, must be ranked under this name, NATURE."

What Emerson considers as the ME, the mental and spiritual personality, is not a fixture. Our personalities like our bodies, grow from infancy to maturity, and may in some cases return again to an infantile senility. Oliver Wendell Holmes said in "The Chambered Nautilus,"

Build thee more stately mansions, O my soul.

And there is no doubt but that most human beings show great development, both mental and spiritual.* Coincidentally, the atoms and molecules comprising our bodies are undergoing continual change, and even the basic patterns of our physical frame are by no means constant. Gradually, we feel our age—and look it. The mental and spiritual possibilities in the brains of most of us are sometimes pitifully limited by physical, chemical, and environmental conditions. Though all service ranks the same with God, as Browning put it, mankind is often slow in recognizing, much less in emulating, even outstanding example and superlative service in individuals. Contemporaries are often poor judges of values. It takes courage to follow the judgment of one's own conscience. Jesus of Nazareth, whose teachings pilloried the brutal materialism of imperial Rome, suffered crucifiction, a Roman form of punishment,[12] by Roman soldiers, under a Roman Procurator. But the Golden Rule based on the Law and the Prophets and emphasized by Jesus, remains the ideal of great numbers of human beings.

Science recognizes that matter is real and indestructible, even though its ultimates are unknown; and similarly we are led to the

* In a paper entitled "Natural Selection and the Mental Capacities of Mankind" (*Science*, 1947, 105, 587-90) Th. Dobzhansky and M. E. Ashley Montagu state: "The genetically controlled plasticity of mental traits is, biologically speaking, the most typical and uniquely human characteristic." Man is extremely adaptable, whereas "the behavior of an individual among social insects is remarkable precisely because of the rigidity of its genetic fixation."

view that mind, or spirit, or soul (the exact word is unimportant) is also real and indestructible, even though we cannot define it, plumb its depths, or understand the basis of its obvious interrelations with matter.

Sir Edwin A. Adrian (Nobel prize, 1932) has stated: "The nervous system is a mass of living cells which has the extraordinary property of appearing to influence, and to be influenced, by the mind . . . It is a material system somehow responsible for such non-material things as emotions and thoughts. These are in a category outside the range of mechanical explanation, and for this reason the working of the nervous system will never be fully explainable in terms of physics and chemistry." This parallels the view of Lord Balfour that "No man can either perceive or imagine the mode in which physiological changes give birth to psychical experiences." But the fact remains that they *do*.

The idealistic philosophy of George Berkeley, Bishop of Cloyne (1684-1753), was based on the psychological principle that, for all sensible things, *being* in reality is identical with *being perceived* (the *esse* of things is *percipi*). Though all material existences have their reality only in some concious mind, human souls have been endowed by God with a substantive existence. To save his system from being entirely subjective, Berkeley added the view that the material universe has a permanent being in the Divine mind. There is a story that one day Bishop Berkeley found the following limerick pinned on a tree in the University quadrangle:

A young Oxford man remarked: "God
Must think it exceedingly odd
That this sycamore tree
Continues to be
When there's no one about in the Quad."

On the following morning, the philosopher's reply was found pinned below:

Dear Sir: Your bewilderment's odd:
I'm always about in the Quad;
So this sycamore tree
Will continue to be
Since observed by, Yours faithfully, God.

Somewhat different versions are given in "The Oxford Book of Light Verse," and in the "Peter Pauper Book of Limericks."

Spinoza's attempt to escape from this dilemma by regarding matter and spirit as aspects of unity still leaves us in ignorance of this all-embracing unitary ultimate. Robert G. Ingersoll, the great agnostic, wrote: "Our ignorance is God; what we know is Science." But the separation of knowledge from ignorance demands successful mental effort, and assumes a mind capable of this. Furthermore, as we enlarge the frontiers of our knowledge, we correspondingly enlarge the frontiers of our ignorance, thus coming more and more into contact with the unknowns and unknowables which Ingersoll termed God.

EVENSONG

However vast our strength or frame,
Or great our power, our wealth or name,
Lord, You are Father of us all,
And we are but Your children small.

Like curious children, we explore
And open many a wond'rous door—
To other doors, but not our goal,
The master-mystery of soul.

With child-like faith, without a care,
At eve we quietly prepare
Ourselves in dreamless sleep to lay,
Until You waken us next day.

REFERENCES

[1] Hackh's "Chemical Dictionary" defines it thus:

"Within the atom it is meaningless to define both the position and the velocity of the electron, because Δx, the error of specifying the velocity of x, multiplied by Δp, the error in specifying the position p, is always of the order of magnitude of Planck's constant, h; that is $x \times p \sim h$. Hence, assuming a definite velocity, only approximate position is possible; likewise, assuming a definite position, only approximate velocity can be calculated."

[2] Charles Galton Darwin, Presidential Address before the Section on Mathematical and Physical Science, British Association for the Advancement of Science, *Science* (1938) 88, 155-160.

[3] Bertrand Russell, *Atlantic Monthly*, August, 1930.

[4] On this question there is an interesting paper by Professor Edward Kasner (Columbia University) in *Scripta Mathematica* (January, 1938) entitled "New Names in Mathematics." Kasner defines a *googol* as 10 to the 100th power, and a *googolplex* as 10 to a power represented by a googol. This latter number is so great that we could go out to the farthest star and then make a tour of the nebulae, writing down zeros all the way in an attempt to express it. (What a few mathematical signs can

do!) Professor Kasner thinks that a suspended book might move upward toward the hand holding it within a googolplex of years, or within some other larger finite number of years. Owing to the inertia-time factor, I still remain a "doubting Thomas."

[5] See, e.g., "The Dawn of Conscience," by Professor James H. Breasted (1932), and "Moses and Monotheism," by Dr. Sigmund Freud (1939).

[6] Dr. Paul Epstein, *Scientific Monthly*, July, 1937.

[7] What differences identical twins do show are probably attributable to differences in cytoplasmic inheritance; for though gene duplication is normally precise, there is, even in identical twins, probably some difference in the apportionment of the cytoplasm of the zygote during the first (and subsequent) mitotic divisions, and this involves particulate inclusions in the cytoplasm.

[8] F. C. Carr, *Chemistry and Industry* (1934) **53**, 123.

[9] *Science*, April 18th, 1924, p. 349 (The Contributions of China to the Science and Art of Medicine).

[10] A. W. von Hofmann, *Berichte* (1884) **17**, 637, referate.

[11] The British Museum has a unique copy, published in London, 1666; but from memoranda left by Wigglesworth, it is possible that undated copies owned by several American historical societies may represent an earlier American edition about 1662.

[12] Cicero said in an oration against Verres: "*Facinus est cruciare civum Romanum; scelus verbere; prope parricidum necare; quid dicam in crucem tollere?*" (It is a wrong to harm a Roman citizen; a crime to beat him; practically parricide to kill him; but to crucify him, what shall I call it?).

Author's Note

My interest in minerals, plants, and animals goes back to my early boyhood. When I entered the College of the City of New York in 1891, it was my good fortune to be introduced to physics and chemistry by Professor Robert Ogden Doremus, and to botany, zoology, geology and paleontology by Professors Ivan Sickels and William Stratford. My thanks are due to these teachers and their assistants, who made science so interesting that my initial leanings toward it were quickened and extended. The fact that my college courses were very broad rather than specialized gave me a wider point of view than I might otherwise have acquired; so that when I graduated in 1896 and took up technological work instead of being able to realize an ambition to become a physician, I was able to continue my scientific studies alone.

In the spring of 1899 I submitted for my degree of Master of Science a thesis* entitled "The Importance and Trend of Recent Work on the Chemistry of Life and the Products of Life," which indicates my early interest in the matters dealt with in this volume—an interest excited largely by the then recent (1895) isolation of zymase from yeast by Buchner. The opening paragraph of this thesis, with a few others, are given below. These views were advanced fifty years ago, on the basis of some of the comparatively slim evidence then available.

"Recent work on the chemistry of life, and its products, has thrown much light upon vital or physiologic phenomena, and has given us no uncertain insight into the nature of life itself. I intend to review briefly part of this work, taking up first ferments and enzymes, and the nature of their action. The discussion of these cell-products leads the way to the second topic—cells and microorganisms, and the nature of their life. . . .

"Buchner's discovery (of zymase) is strong and almost conclusive evidence in favor of the view of Berthelot and Hoppe-Seyler [that organisms produce chemical substances which cause fermentation], and is of the utmost importance because it helps to break down a false barrier which had been erected between physiology and chemistry. Physiological laws are chemical laws, however great their complexity; and matter is bound by these same laws, be it organic or inorganic, living or dead. As a vital function fermentation must always be mysterious and inscrutable, while as a chemical process it is susceptible of explanation.

"It appears then that all fermentations are purely chemical changes brought about by substances which usually are highly complex and highly nitrogenous, as analysis shows. To confirm this assumption more fully, work should be done along the lines laid down by Buchner, and the active enzymes should be isolated from all fermentative microorganisms. In the meanwhile we may examine into the nature of the chemical action that takes place.

"There are numerous reactions which go by the name of continuous processes. The decomposition of the diazo-compounds by cuprous salts (Sandmyer's reaction) is a case in point. It proceeds according to the equation

$$C_6H_5N:N:R + Cu_2R_2 = C_6H_5R + N_2 + Cu_2R_2$$

so that the process is theoretically continuous. The cuprous salt acts the part of a carrier or "go-between," just as do iodine, ferric chloride, aluminum chloride and

* A short excerpt from this unpublished thesis appears in a paper written jointly with Dr. Calvin B. Bridges, entitled "Some Physico-Chemical Aspects of Life, Mutation, and Evolution," published in Vol. II, of series "Colloid Chemistry, Theoretical and Applied," edited by Jerome Alexander, Reinhold Publishing Corp., N. Y., 1928.

many other substances under different circumstances. The catalytic decompositions of hydrogen peroxide and hypochlorous acid . . . are apparently continuous processes, although the intermediate compounds have not been determined.

"Not alone can a small quantity of one substance decompose a large quantity of another, but the same substance yields varied products depending on the nature of the go-between. Ethyl acetoacetate, when hydrolyzed with dilute boiling acids, yields ketones; while if strong alcoholic potash be employed, acids are produced. This latter is not a continuous process because the potash is eliminated by combining with the acid produced; but it illustrates the point.

"Now I believe that the actions of diastase, and in fact of all other enzymes, are in the nature of continuous processes. I base this, as yet, almost entirely upon analogy, but can indicate the line of experimentation necessary to confirm the theory:

(1) Active enzymes must be isolated, and their chemical constitution and structure investigated.

(2) The composition of the substances to be decomposed must be understood, and also the nature of the compounds they can form with the structurally determined enzymes. . . .

"The enzymes and enzyme-like toxins we have just been considering occur only in, or as the products of, living cells. What is their relation to the life of the cells? Do they throw any light upon the actual nature of life itself? After carefully considering the question, it appears to me that life is an enzyme-like continuous chemical process, or rather a series of such processes. Among the facts that led to this conclusion, a few may be discussed: attenuation, immunity, antisepsis and soil nitrification. . . .

"Enzymic and cell-metabolic processes are so completely dependent upon chemical conditions that they are without doubt purely continuous chemical processes. True, the changes which take place are of great, almost appalling complexity; but they need not overcome us. The sugars, once regarded as of almost unknowable complexity, are now known as a series of hydroxy derivatives of normal hexane. . . ."

The scientific literature shows an ever-increasing realization of the importance of catalysis in life processes. It must be remembered that enzymes are biocatalysts, and that antibody formation occurs catalytically, as indicated in my Protoplasma paper (1931) and in the section on "Immunology and Serology" in the fourth edition (1937) of my book "Colloid Chemistry" (D. Van Nostrand Co.). In 1936 Dr. Karl Landsteiner was kind enough to review with me the manuscript of this section, as indicated on p. 413.

Dr. Alwin Mittasch, well known for his work in industrial catalysis, has recently published the following books (J. Springer, Berlin): "Ueber katalytische Verursachung im biologischen Geschehen" (1935), "Katalyse und Determinismus" (1938), and "Kurtze Geschichte der Katalyse in Praxis und Theorie" (1939). A still later book is now on press, entitled "Lebensproblem und Katalyse" (J. Ebener-Nerlag, Ulm/Donau).

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